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Final Report

HEAVY METAL REMOVAL FROM GOLD MINE TAILINGS BY INDIGENOUS PLANTS (OLDHAM PHASE II)

Project No. E-257

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Project No. E-257

Prepared for

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EXECUTIVE SUMMARY

Mobilization of heavy metals from mine tailings into surface and groundwaters is a persistent environmental problem at both active and abandoned mines. Gold mines frequently have additional contamination problems resulting from the use of mercury or cyanide during the extraction process. Because these toxic metals may be leached from the site for decades or even centuries, innovative means of treating runoff waters are urgently required to prevent further contamination of downstream environments.

A biotechnology approach to this problem has been utilized in many locations around the world. Indigenous species of plants are cultured on-site in order to (a) bioaccumulate metals from surface and ground waters, (b) reduce erosive loss of contaminated soils and tailings, (c) mechanically trap fine, metal-rich particles, and (d) stimulate soil microbial communities to produce metal-binding sulfides and volatile metallo-organic compounds. The success of this approach has been quite variable, principally because of a lack of understanding of plant ecology and the biogeochemical processes controlling transport and uptake of heavy metals.

A two-phase study of an abandoned gold mine site near Oldham, Nova Scotia, was initiated in 1988, with an ultimate goal of determining the feasibility of using indigenous plants as "biological filters" for mercury (Hg), arsenic (As), and other heavy metals. Phase I studies indicated that most plant species were very tolerant of heavy metals, and accumulated as much as 10,000 ppm As and 16 ppm Hg in their tissues. As early colonizers of the tailings, a moss species (*Campylium stellata*), a stonewort (*Chara globularis*), the water horsetail (*Equisetum fluviatile*), a rush (*Juncus articulatus*), and the broad-leaved cattail (*Typha latifolia*), were chosen for further lab and field investigations of their utility in biotechnology applications. Commercially available grass and legume seed were also tested for their abilities to survive and bioaccumulate metals from the Oldham tailings.

Laboratory experiments suggested that the rush and several grass species, most notably Red Top (*Agrostis gigantea*) and Creeping Red Fescue (*Festuca rubra*), would be the best candidate species for use in treating contaminated runoff from gold mine sites. Addition of a high-phosphorus fertilizer and lime are also recommended as part of a reclamation scheme for bare, exposed tailings. Concentrations of As and Hg in the shoots and roots of lab-grown plants approximated those in plants that were transplanted to the field for five- and ten-week incubations, and those from samples collected as part of Phase I vegetation surveys. As noted in earlier field studies, water availability was the key determinant of the success of transplants in retaining heavy metals on-site. Summer drought killed off many of the transplants as well as much of the indigenous vegetation. Clearly, control of water-level must be an integral part of any biological treatment strategy.

The fact that heavy metals are still being mobilized from the main tailings deposit, despite an almost complete vegetation cover, suggests that simple additions of more vegetation, or replacement with species that accumulate large quantities, will not greatly reduce the loss of heavy metals from the site. A "constructed wetland" or organic compost-based approach may be more preferable means of treating metal-contaminated waters from mine sites.

RÉSUMÉ

La contaminations des eaux souterraines et de surface par les métaux lourds provenant des résidus miniers est depuis longtemps un problème environmental autant pour les mines actives qu'abandon-nées. Ce problème est plus aigu pour les mines d'or, à cause des procédés d'extraction utilisant du mercure et du cyanure. Parce que le lessivage de ces substances néfastes peut se poursuivre pendant des décennies, parfois, des siècles, il importe de développer de toute urgence des méthodes innovatrices pour traiter les eaux d'écoulement afin de prévenir la contamination des régions situées en aval des sites miniers.

La biotechnologie ouvre de nouvelles avenues déjà en usage en certains endroits. Des espèces de plantes indigènes sont cultivées afin: a) de bio-accumuler les métaux lourds des eaux de surface et souterraines, b) de réduire l'érosion des dépôts miniers, c) de filtrer mécaniquement les fines particules enrichies en métaux, d) de stimuler la croissance des bactéries du sol produisant des sulfures à liens métalliques et des composés volatiles métallo-organiques. Les résultats de ces mé-thodes ont été très variables, principalement à cause d'un manque de compréhension de l'écologie des plantes et des processus biochimiques controllant l'échange et le transport des métaux lourds.

Une étude en deux phases d'un site minier aurifère près de Oldham, Nouvelle-Ecosse, a été entreprise en 1988. Le but de l'étude était d'évaluer la possibilité d'utiliser des "filtreurs biologiques" constitués de plantes indigènes pour capter le mercure (Hg), l'arsenic (As) et d'autres métaux lourds. Les résultats de la phase I ont démontré que la plupart des plantes sont tolérantes aux métaux et peuvent retenir jusqu'à 10,000 ppm de As et 16 ppm de Hg dans leurs tissus. Les espèces suivantes furent choisies afin d'expérimenter leur application biotechnologique pour la colonisation hative des sites de dépôt miniers: des mousses (*Campylium stellata*), des algues macrophytes (*Chara globularis*), des prêles (*Equisetum fluviatile*), des joncs (*Juncus articulus*), et des quenouilles à longues feuilles (*Typha latifolia*). Des semences d'herbes de pâturage et de fèves disponibles commercialement furent aussi testées afin d'établir leur résistance aux métaux lourds et leur capacité de bio-accumulation de ces métaux.

D'après les expériences en laboratoire, les joncs et plusieurs espèces d'herbes de pâturage, notamment le foin follette (*Agrostis gigantea*) et le fétuque rouge (*Festuca rubra*), émergent comme étant les plus efficaces pour traiter les eaux contaminées de dépôts miniers aurifères. L'addition de fertilisants riches en phosphore et en lime est recommandée dans le cas des dépôts de résidus miniers directement exposés aux intempéries. Les concentrations de Hg et de As dans les pousses et les racines des plantes cultivées en laboratoire sont proches de celles observées dans les plantes transplantées en nature et laissées en incubation pendant cinq à dix semaines. Il en est de même des échantillons recueillis durant la campagne d'échantillonnage de la phase I. Tel que mentionné pendant les études précédentes, le facteur clef du succès ou de l'échec des transplantations a été la présence ou le manque d'eau. Les sécheresses d'été ont détruit plusieurs transplants ainsi qu'un partie de la végétation indigène. Le contrôle du niveau d'eau fait donc partie intégrante de la stratégie de traitement biologique.

Malgré une couverture presque complète des dépôts de résidus miniers par la végétation, des métaux lourds s'échappent encore; cela indique que l'ajout de plus de vegetation ou le remplacement de la végétation existante par des plantes ayant une plus grande capacité de rétention des métaux ont peu de chance d'améliorer significativement le traitement des eaux d'écoulement. Nous recommandons plutôt d'étudier l'alternative de "développer un marais" ou d'utiliser un filtre de compost pour traiter les eaux contaminées par les métaux lourds.

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1. INTRODUCTION

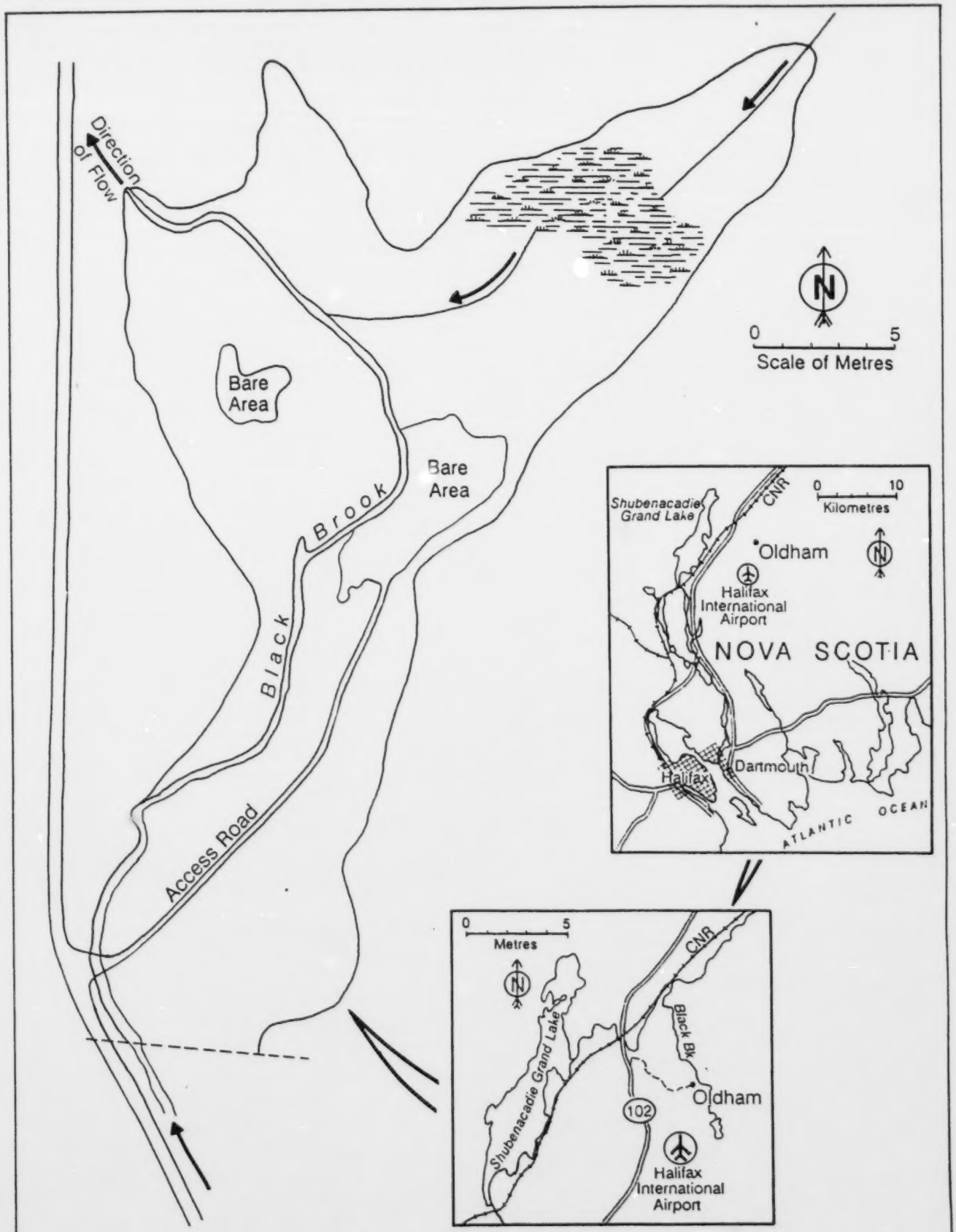
1.1 Background Information

Heavy metal contamination of soils, water and biota near mining sites is a direct consequence of natural weathering of these elements from exposed rock surfaces and waste tailings piles. This contamination process is accelerated by acidic waters that result from acid rain and the biogeochemical oxidation of pyritic materials associated with the ore deposits. Most heavy metals are more soluble in acidic waters. Unfortunately, leaching of heavy metals and generation of acidic runoff may continue for decades or even centuries into the future. Gold mining sites frequently have additional contamination problems resulting from the use of mercury or cyanide in the extraction process. The challenge of the 1990's will be to develop innovative means of treating runoff waters and eliminating contamination of downstream environments.

Gold mining practises in Nova Scotia between the mid 1800's and early 1900's, employed considerable quantities of mercury to amalgamate the relatively high-grade ores found at many of the discovery sites. Gold bearing ores were crushed to a sandy consistency, mixed with water and elemental mercury, and the resultant slurry passed over mercury-coated amalgam tables to extract the gold. Waste tailings, together with non-reclaimable mercury, were then dumped near the mills, typically into nearby stream beds. Fifty years later, mercury, arsenic and other heavy metals are still being leached from these abandoned sites. Plants and animals living nearby have accumulated these metals, often to levels that impair life-sustaining processes (Brooks et al., 1982; Dale and Freedman, 1982; Mudroch and Clair, 1985; P. Lane and Associates Limited, 1986).

Because of impending plans to re-work some of the abandoned tailings deposits for residual gold, P. Lane and Associates Limited was commissioned to undertake a two phase study to (i) characterize the water, soil geochemistry, and vegetation communities at one of the proposed sites, near Oldham, Nova Scotia (see Figure 1-1), and (ii) investigate the feasibility of using indigenous plants to stabilize the tailings and remove mercury and arsenic from waters draining the tailings deposits.

Results of Phase I investigations indicated that heavy metal mobilization from the site is a function of both physical-chemical and biological processes (Lane et al., 1988; 1989). Successful establishment of vegetation communities and accumulation of organic-rich soils on top of the tailings appears to (a) reduce erosive loss of contaminated soils and tailings, (b) mechanically trap fine, metal-rich particles, (c) adsorb and accumulate dissolved metals, and (d) stimulate soil microbial communities (which in turn produce metal-binding sulfides and volatile metallo-organics). Most plant species living on the tailings were apparently very tolerant of arsenic and mercury, and grew throughout the entire range of metal concentrations (70 to 26,000 ppm arsenic and 0.7 to 8.9 ppm mercury). The principal determinants of plant community composition around the site were: depth to water table, soil organic matter content, and soil bulk density.



The Abandoned Gold Mining Site at Oldham, Nova Scotia

P. LANE AND ASSOCIATES LIMITED

FIGURE 1-1

That some of the dominant plants were able to accumulate large concentrations of arsenic and mercury, up to 10,000 ppm arsenic and 16 ppm mercury, suggested that indigenous plants may be useful as "biological filters" for treatment of runoff waters and tailings reclamation. This ability to accumulate large quantities, often with little apparent reduction in growth or reproduction rates, is widespread among plants inhabiting mine sites (e.g. Porter and Peterson, 1977; Lee et al., 1981; Fayed and Abd-El-Shafy, 1985; Harborne, 1988; Kelly, 1988). Although most of these plants are believed to have developed a genetic resistance to high concentrations (Macnair and Cumbes, 1987; Collard and Matagne, 1990), some species only have a physiological tolerance to high metals and gradually lose this ability when grown in the absence of the metal (Stokes and Dreier, 1980).

Until recently, many investigators felt that if sufficient numbers of these metalliferous plants were planted at contaminated sites, metals in surface runoff waters could be effectively scavenged by the plants and retained on-site. This "biotechnology approach" to a common environmental problem has garnered considerable success in many United States locations (Wolverton, 1975; Wolverton and McDonald, 1975; Bishop and DeWaters, 1988). Although only limited research has been carried out at Canadian sites, the biotechnology approach has been shown to be very effective treatment scheme for removal of some metals from mine wastewaters (Kalin, 1989; 1990; Smith and Kalin, 1990). Calculations of the actual percentage of metals removed by the plants, however, reveal less than 1% removal into the shoots and roots of plants (Sencindiver and Bhumbla, 1988). Most of the metals appeared to be bound to biofilms (algae and bacteria) living on the roots and shoots of the plants (Bishop and DeWaters, 1988). The bottom sediments were the principal "sink" for most of the heavy metals. Experimental studies conducted by researchers from the University of Waterloo and Noranda Inc. at several eastern Canadian sites further demonstrated that groundwater flow beneath vegetation covers and seepage into streams may be the most important factor governing the loss of heavy metals from tailings deposits (Veldhuizen et al., 1987). At the locations examined, vegetation covers were not effective in reducing water infiltration or losses of heavy metals from tailings sites.

1.2 Objectives of the Present Study

In order to investigate the feasibility of using indigenous plants to scavenge and retain mercury, arsenic and other heavy metals from waters draining an abandoned gold-mining site, three laboratory experiments and one field experiment were conducted using some of the dominant plants found at the Oldham site (Figure 1-1). Based on the results of Phase I studies and published data from other sites in Canada and abroad, these experiments utilized a moss species *Campylium stellata*, a stonewort *Chara globularis*, the water horsetail *Equisetum fluviatile*, a rush *Juncus articulatus*, broad-leaved cattail *Typha latifolia*, alsike clover *Trifolium hybridum*, birdsfoot trefoil *Lotus corniculatus*, and four species of grass (red top *Agrostis gigantea*, creeping red fescue *Festuca rubra*, reed canary-grass *Phalaris arundinacea*, and Reubens Canada bluegrass *Poa compressa*). The latter six species were all grown from commercially-available seed, and are commonly used for tailings reclamation (N.K. Davé, CANMET-Elliot Lake Laboratories, pers. comm.; Gadgil, 1969; LeRoy and Keller, 1972; Smith and Bradshaw, 1979). All other species

were collected from the site and cultured in the greenhouse at Dalhousie University, Halifax, Nova Scotia, for several months prior to initiation of the lab and field experiments. During the acclimation period, the plants were maintained in pots or trays containing an artificial soil-mix (Pro-Mix), or for *Chara*, streamwater from Black Brook. Water levels were maintained by an automatic sprinkler system, periodically augmented by hand-watering.

The first two lab studies were designed to test the effect of contaminated waters on growth of plants taken from the Oldham site, and to determine which species, if any, might be successfully used for biotechnology applications at gold mining sites. Although not in the original "Terms of Reference" for this contract, we added a third lab study to examine the effect of liming and different fertilizers on the growth and metal uptake potential of grass and legume plants that are typically used for tailings reclamation projects. Grass species were chosen because they grow rapidly, provide a good ground-cover, and encourage soil formation and further biological succession. The legumes, together with microbial symbionts, fix atmospheric nitrogen, thereby enhancing the fertility of developing soils. Finally, the field experiment attempted to demonstrate the feasibility of (i) transplanting the laboratory-acclimated plants, simulating plants from nearby non-contaminated sites, to a tailings site, and (ii) using plants as "biological filters" for alleviating mercury and arsenic transport from gold mining sites.

2. METHODS AND MATERIALS

2.1 Laboratory Experiment #1

This first experiment examined the effects of arsenic, mercury, and a combination of the metals, on the growth of a moss *Campylium stellata*, a stonewort *Chara globularis*, the water horsetail *Equisetum fluviatile*, a rush *Juncus articulatus*, and the broad-leafed cattail *Typha latifolia*. The metal concentrations used in this experiment approximated the average values documented at the Oldham site (~100 ppm arsenic and 1 ppm mercury; made from stock solutions of sodium arsenate and mercuric chloride). Known quantities of plants or rhizomes from the greenhouse stock cultures were placed in 1 L Mason jars that contained silica sand (Nova Scotia Sand & Gravel #0; see Appendix 1 for grain size characteristics), and (a) a hydroponic nutrient solution (Hoagland's solution; Hoagland and Arnon, 1950) amended with arsenic, (b) Hoagland's solution plus mercury, (c) Hoagland's solution with both arsenic and mercury, and (d) Hoagland's solution alone (control treatment). Solution level was maintained at the sand-air interface with periodic additions of distilled water. Five replicate jars were used for each treatment (random allocations within the growth room).

After seven weeks growth under fluorescent lights (12 hour photoperiod; ~100 μEinsteins/m^2/s irradiance) at 22°C, the plants were harvested, weighed for fresh and dry weights, and portions analyzed for metal contents. Root and shoot concentrations of metals were determined whenever possible. It is now well known that plant surfaces that are constantly exposed to metal-enriched waters, such as the roots, often have much higher tissue concentrations of heavy metals than unexposed, above-ground portions of the plant.

Samples of dried root and shoot samples were digested in concentrated nitric acid, and then analyzed for arsenic and mercury content by standard geochemical techniques at the Minerals Engineering Centre of the Technical University of Nova Scotia in Halifax. Standard reference vegetation samples (U.S. National Bureau of Standards #1571 - Orchard Leaves) were also analyzed in order to quantify the quality assurance/quality control (QA/QC) for the lab methods (results are summarized in Appendix 2). Although only five standard reference vegetation samples were analyzed, arsenic and mercury seemed to be quantitatively extracted and measured by methods used by staff of the Minerals Engineering Centre.

2.2 Laboratory Experiment #2

Considering the large range in metal concentrations observed in soils from the Oldham site (70 to 26000 ppm arsenic and 0.7 to 8.9 ppm mercury), the second experiment was designed to test the ability of the species that exhibited the best growth and metal accumulation potential in Experiment #1 to accumulate metals under a variety of different metal regimes. The methodology was also changed from that used in earlier studies in order to more closely mimic field conditions; the hydroponic nutrient solution and stock metal amendments were replaced with an artificial soil mixture (Pro-Mix) and actual tailings from

the Oldham site. Tailings were collected from a location known to have high concentrations of both arsenic and mercury.

Known quantities of the rush (*Juncus articulatus*) were transplanted into plastic, ½ gallon pails containing a 3 cm artificial soil layer (approximating the typical soil cover at Oldham), overlying ~10 cm of either Oldham tailings, three mixtures of tailings and silica sand (75% tailings:25% sand, 50:50 and 25:75 mixtures), or just silica sand (the grain size distributions were nearly identical in these mixtures, see Appendix 1). Concentrations of arsenic and mercury, together with iron, manganese, aluminum, cadmium, copper, lead and zinc in these substrates, are summarized in Appendix 3. Each bucket was initially filled to the soil-air interface with water from Black Brook (via a filling tube leading to the bottom of the bucket), but was subsequently maintained with tap water as required.

Measures of plant growth rates and tissue burdens of arsenic, mercury, iron, manganese, aluminum, cadmium, copper, lead and zinc were then determined in the 25 experimental pails (five replicates of five treatments) following seven weeks incubation at 22°C under a 12 hour photoperiod (light intensity ~100 μEinsteins/m^2/s). As outlined above, dried portions of the roots and shoots were digested with nitric acid prior to geochemical analyses. The QA/QC determinations for the additional metal analyses are also summarized in Appendix 2. Except for iron and cadmium, all metals were quantitatively recovered by methods used by staff of the Minerals Engineering Centre (aluminum values for the NBS-orchard leaves were not provided). Iron concentrations appeared to be low by about 50 %, while cadmium values were approximately two times higher than the certified values (by comparison, all metals in tailings samples were quantitatively extracted, unpublished data). Plant tissue concentrations reported herein have not been adjusted to reflect these analytical differences.

2.3 Laboratory Experiment #3

The final lab experment was designed to examine how six commercially-available grass and legume species (red top *Agrostis rubra*, creeping red fescue *Festuca rubra*, reed canary-grass *Phalaris arundinacea*, Canada bluegrass *Poa compressa*, alsike clover *Trifolium hybridum*, and birdsfoot trefoil *Lotus corniculatus*) would adapt to wet tailings from Oldham. All of these species are known to do well on other types of tailings in Nova Scotia and other areas of Canada. Seeds were spread onto 0.13 m^2 trays containing ~1.5 L of (a) wet tailings that were previously limed to increase the pH from ~4.5 to 7 and supplemented with either a high nitrogen, high phosphorus, or a balanced medium-level fertilizer (30%:10%:10%, 10%:52%:10%, and 15%:15%:18% mixtures of nitrogen: phosphorus:potassium, respectively), (b) wet tailings that only had the three nutrient amendments, and (c) silica sand plus the three nutrient amendments. The tailings were originally immersed with water from Black Brook, but were kept continuously wet by additions of tap water for the six week duration of the experiment. Fertilizer amendments and seeding levels were scaled down from recommended application rates of about 100 kg/ha (10 g/m^2; N.K. Davé, CANMET-Elliot Lake Laboratory, pers. comm.; Halifax Seed Co. Inc., Turf Seed Specification Manual). The plant shoots were subsequently harvested, dried, weighed, and portions assayed for arsenic, mercury, iron, manganese, aluminum,

cadmium, copper, lead and zinc contents (provided sufficient material was available for lab analyses).

2.4 Field Experiments

Cuttings or rhizomes of the five plant species used in Lab Experiment #1 (moss, *Chara*, horsetail, rush and cattail) were also transplanted back to appropriate environments at the Oldham site for incubations of approximately five to ten weeks between July and September, 1989. Sites chosen for the transplants were based on earlier vegetation studies carried out at this site. Twenty replicate samples were transplanted to the sites on July 18. Half of the samples were to be harvested after ca. five weeks; the other ten, five weeks later. Unfortunately, severe drought conditions dried up the pond and drastically lowered the water table. As a result, many of the plants were killed-off (half of the cattails and all *Chara* plants). Other experimental transplants were actively sought-out and destroyed by local residents on recreational vehicles. Because of these problems, five additional replicates of each species were brought to the sites on August 8 for subsequent harvesting five weeks later (only the five moss transplants were recovered though). At the end of the two incubation periods (36 and 66 days), replicate samples were harvested, weighed, and assayed for arsenic and mercury contents (using the same methods as outlined earlier).

3. RESULTS AND DISCUSSION

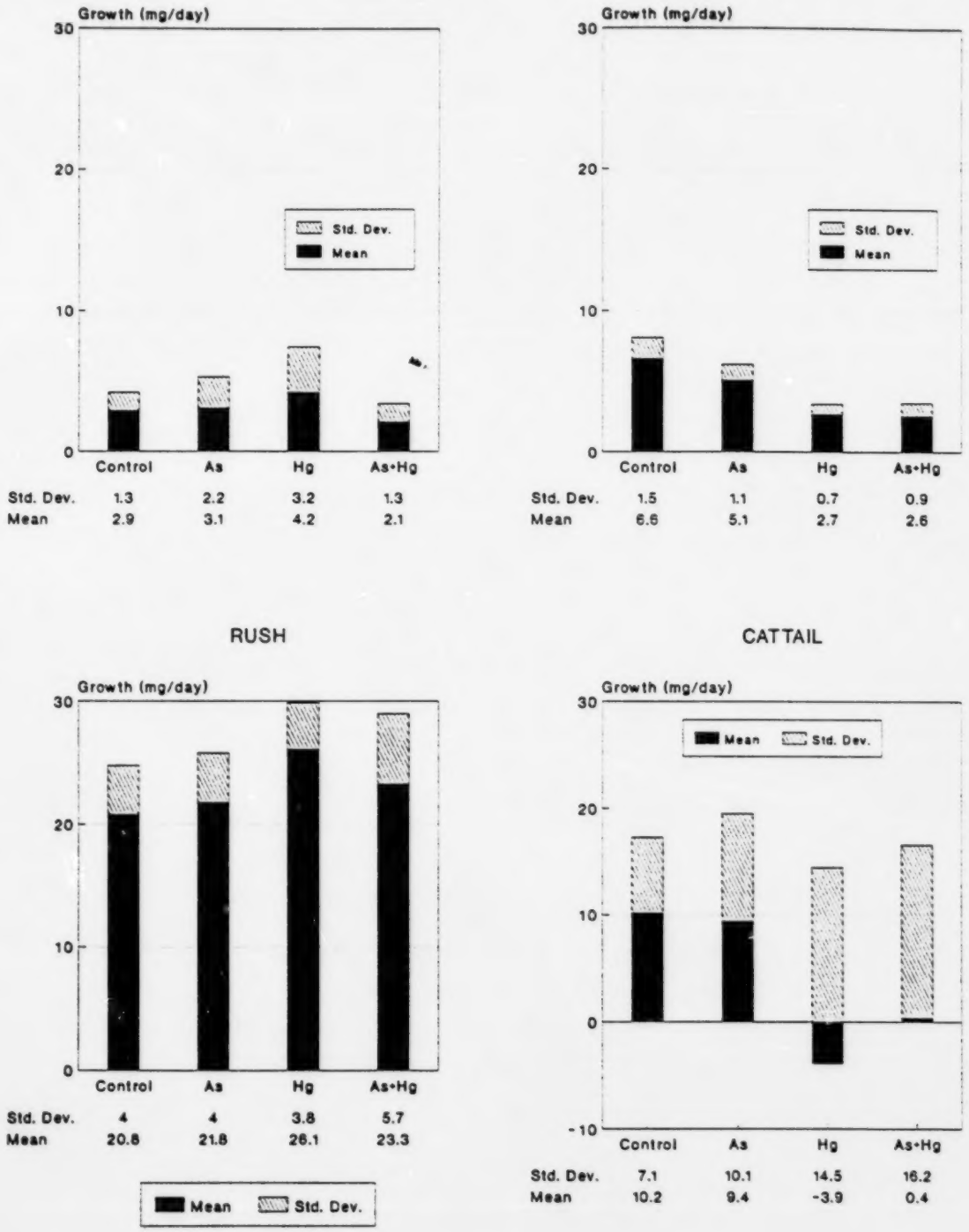
3.1 Effects of Arsenic and Mercury on Plant Growth

All species but *Chara globularis* withstood transplanting procedures and grew well during the seven week-long experiments (see Figure 3.1-1; Appendix 4). Algal contaminants repeatedly covered the *Chara* plants, killing them within a few days. Consequently, further experimentation with this species was suspended. Although there was considerable variability in the measured growth rates of the other four species, only mercury-amended samples and mercury plus arsenic-amended samples appeared to cause any inhibition of plant growth. There was no evidence for synergistic effects of the two metals on either growth rates or metal accumulation (see Figures 3.1-2 and 3.1-3). The lack of growth inhibition by arsenic may be a consequence of high phosphorus levels in the Hoagland's nutrient solution. It is well known that arsenic toxicity can be modified by phosphorus in many types of plants (Planas and Healey, 1978; Macnair and Cumbes, 1987). Adding phosphorus fertilizers to arsenic-rich soils has even been used to enhance plant establishment and resistance to this contaminant (e.g. Benson, 1953).

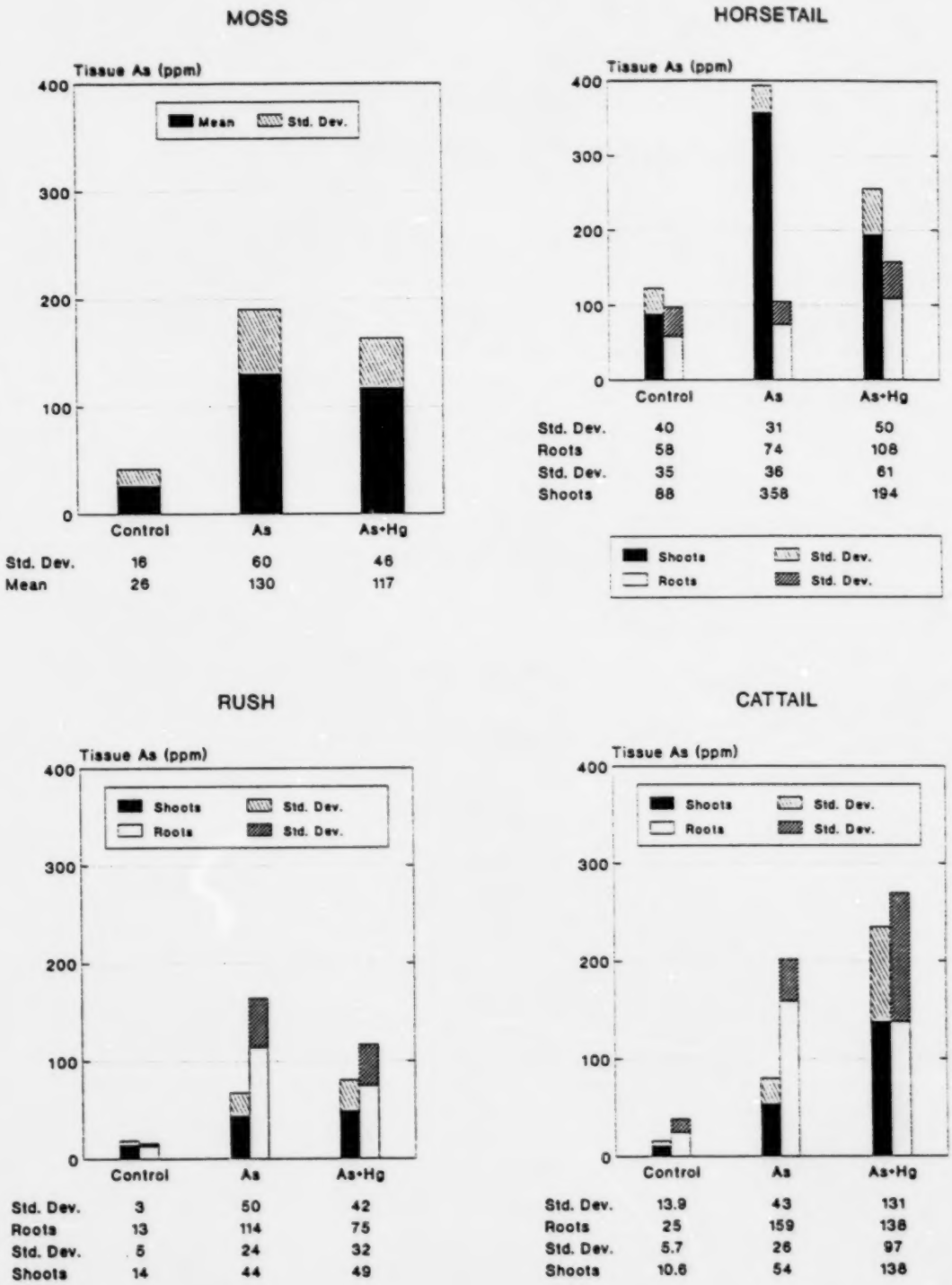
Horsetails and cattails appeared to take up the most metals. Tissue burdens of ~20 ppm mercury (Hg) and 200 ppm arsenic (As) in the shoots and roots were nearly twice those observed for the moss and rush species (see Figures 3.1-2 and 3.1-3; Appendix 5). The growth of both species, however, appeared to be inhibited by added metals, especially by Hg (Figure 3.1-1). Although, this might explain the low abundance of cattails at the Oldham site, it is unclear as to why an apparently sensitive species, the horsetail *Equisetum fluviatile*, would be a prime colonizer of tailings deposits. It thrives in wet through dry conditions, at all levels of Hg and As, and may even be used as an indicator of gold availability (Brooks et al., 1981; Dale and Freedman, 1982). One probable explanation is that the laboratory reagents used here were not representative of the forms of the metals in Oldham soils and tailings. As a consequence, all subsequent experiments were conducted with actual tailings. Horsetails would be an obvious choice here, considering the results of this "preliminary study" and previous field observations. We later rejected this plan because horsetails do not provide an adequate ground-cover for reducing losses of particulate metals from tailings sites, nor a large biomass for dissolved metal uptake.

Mosses, such as *Campylium stellata*, would also be prime candidates for biotechnology applications if they grew faster. Kalin and Buggeln (1986) reached a similar conclusion for two other species of moss (*Drepanocladus fluitans* and *Sphagnum riparium*). Mosses often provide a continuous mat over tailings, effectively stopping erosive loss of particles. They are also early colonizers of moist, barren tailings. In our experiments with *Campylium*, moss growth was not inhibited by either Hg or As amendments. This appears to be a general characteristic of the group.

The rush *Juncus articulatus* was the fastest growing species and incorporated moderate levels of metals. Considering their prominence in semi-aquatic areas at the Oldham site (Lane et al., 1988, 1989), and their demonstrated growth

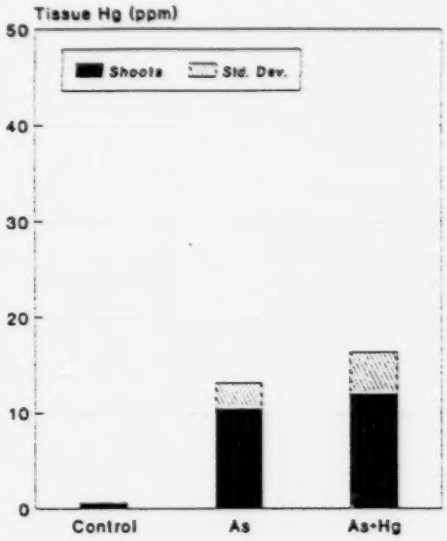


The effect of arsenic and mercury on the growth of the moss *Campylium stellata*; the horsetail *Equisetum fluviatile*; the rush *Juncus articulatus*; and the cattail *Typha latifolia*.



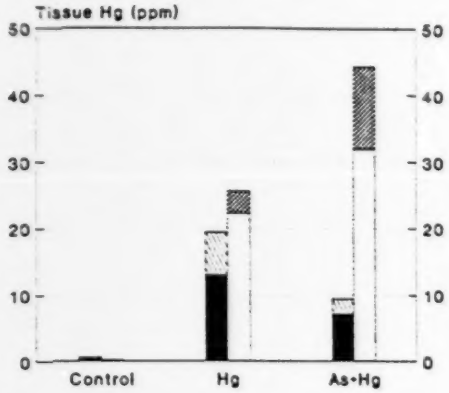
Arsenic concentrations in the shoot and roots of the moss *Campylium stellata*; the horsetail *Equisetum fluviatile*; the rush *Juncus articulatus*; and the cattail *Typha latifolia*.

MOSS



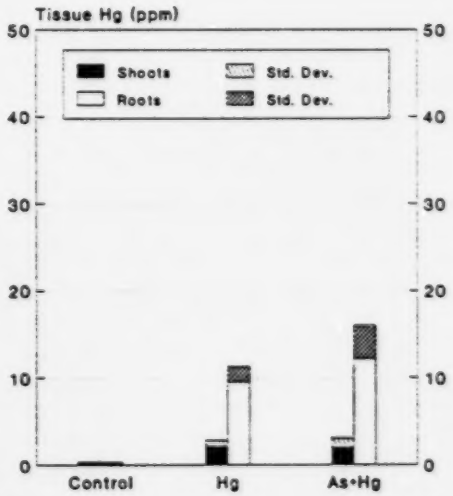
	Control	As	As+Hg
Std. Dev.	0.13	2.75	4.34
Shoots	0.49	10.41	12

HORSETAIL



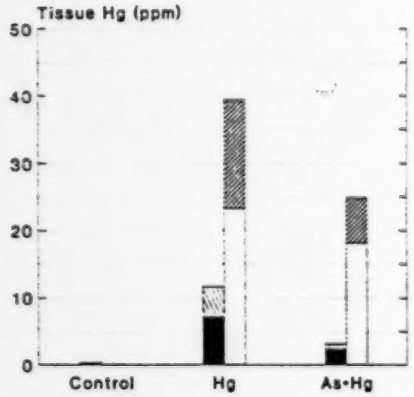
	Control	Hg	As+Hg
Std. Dev.	0.02	3.37	12.46
Roots	0.18	22.22	31.88
Std. Dev.	0.17	6.29	2.26
Shoots	0.47	13.04	7.17

RUSH

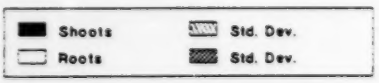


	Control	Hg	As+Hg
Std. Dev.	0.07	1.69	3.85
Roots	0.29	9.59	12.2
Std. Dev.	0.03	0.69	1.06
Shoots	0.39	2.12	2.05

CATTAIL



	Control	Hg	As+Hg
Std. Dev.	0.07	32.2	13.41
Roots	0.14	46.5	36.35
Std. Dev.	0.11	4.41	0.8
Shoots	0.31	7.22	2.41



Mercury concentrations in the shoot and roots of the moss *Campylium stellata*; the horsetail *Equisetum fluviatile*; the rush *Juncus articulatus*; and the cattail *Typha latifolia.*

potential in the lab, we chose this species for further study of their responses to a variety of metal concentrations in mixtures of "clean" sand and Oldham tailings.

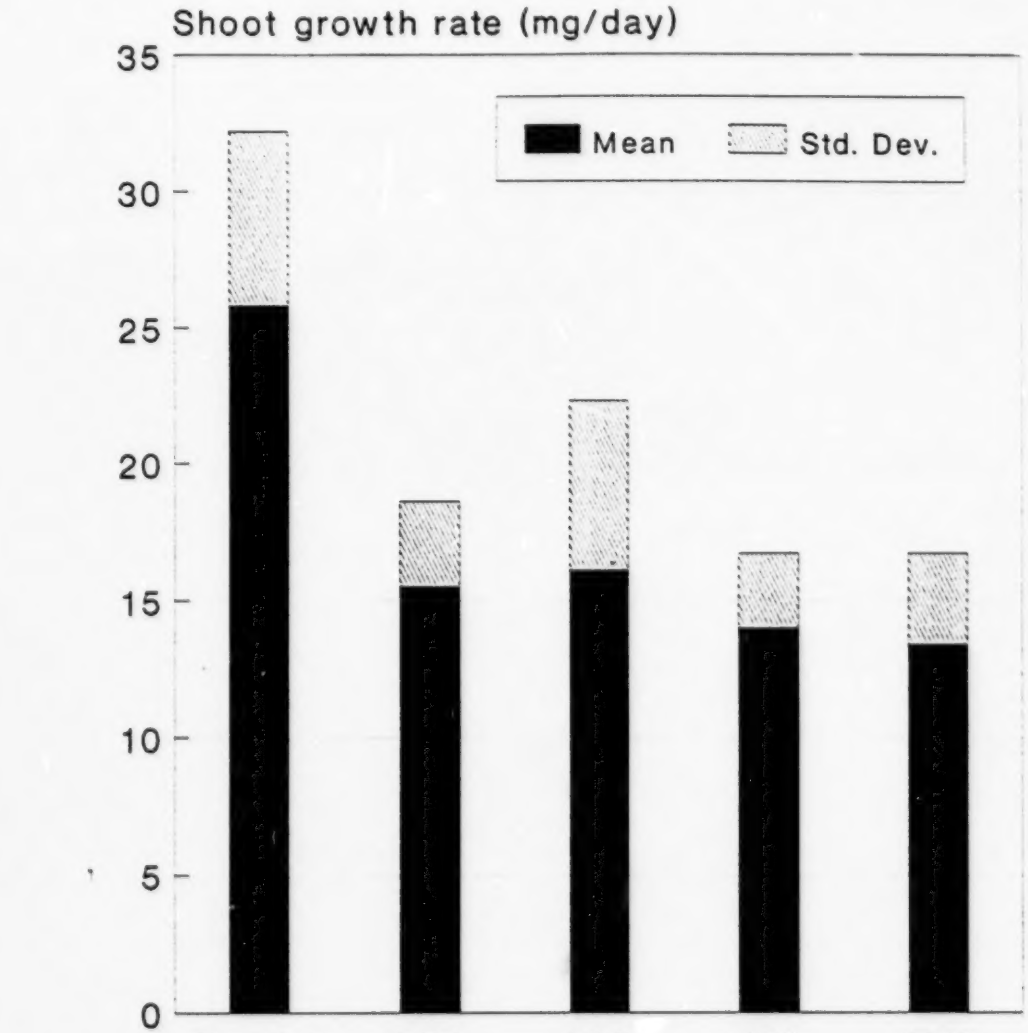
3.2 Effect of Different Concentrations of Metals on the Growth of the Rush *Juncus articulatus*

The rush grew equally well in all of the tailings and tailings:sand mixtures, but overall growth of shoot biomass was reduced by approximately 40% compared to the control pots (see Figure 3.2-1; Appendix 6). Root biomass often decreased during the course of the experiment (perhaps due to our inability to extricate all of the fine roots from the soil and tailings), hence total measures of plant growth were not calculated. Clearly, heavy metal resistance in these plants has some "cost" associated with it. Only a small amount of the limiting substance(s), unknown at this time, appears to be responsible for growth rate suppression. One might hypothesize that differences in soil pH and hardness may be important for alterations in membrane permeability, metal toxicity, and ultimately, growth potential (Harborne, 1988; Kelly, 1988). Fortunately, viability does not seem to be altered; plants were able to grow equally well throughout the range of metal concentrations found in the tailings and sand/tailings samples (2000 to 8000 ppm As, 1 to 5 ppm Hg, 10 to 40 ppm Cu, 50 to 160 ppm Pb, and 30 to 90 ppm Zn; see Appendix 3).

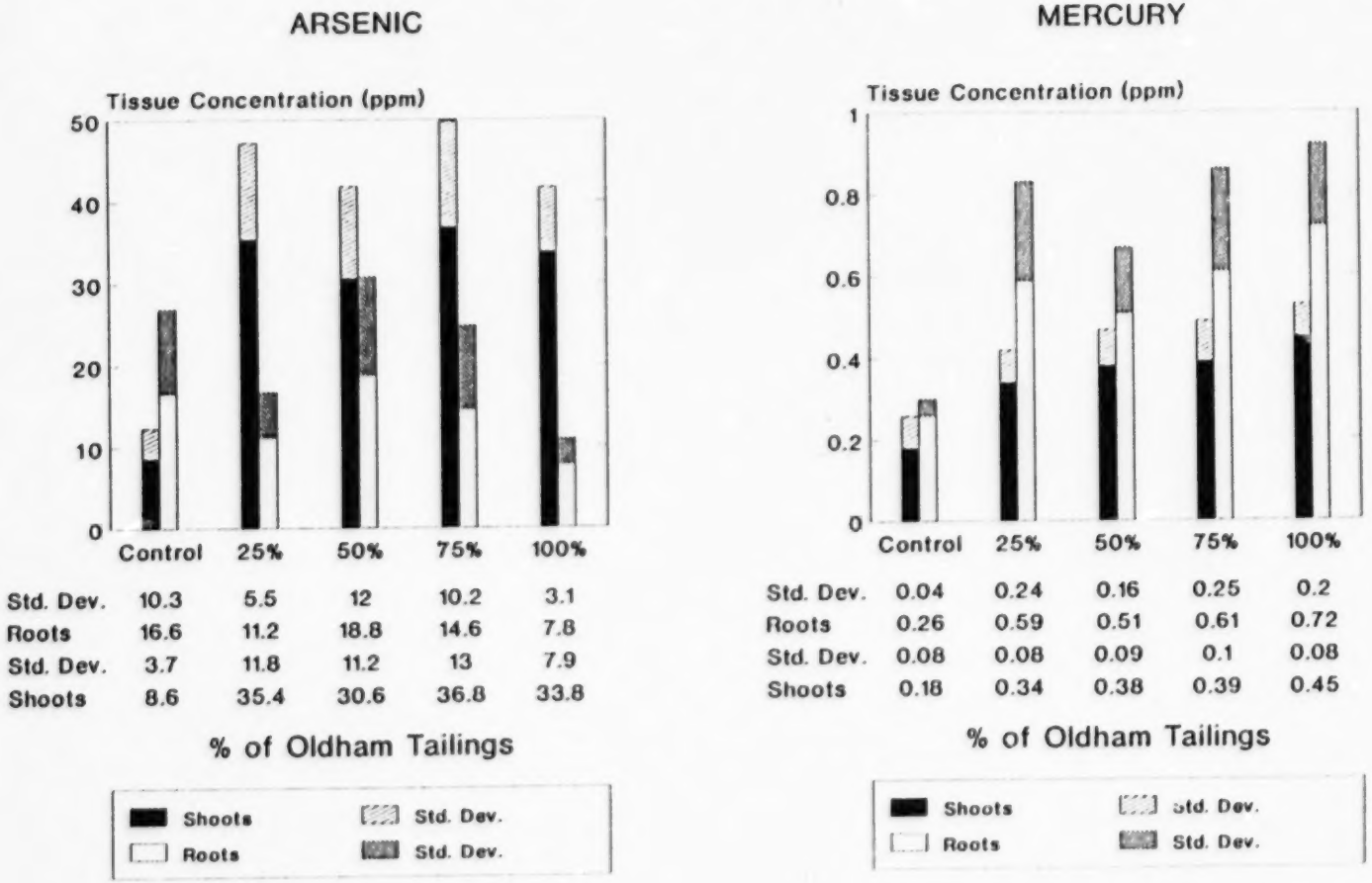
Metal concentrations in shoots tissues compared to those of roots were quite variable depending upon metal examined and quantity available in the tailings or Pro-Mix soil (see Figures 3.2-2 to 3.2-4; Appendix 7). Tissue burdens of arsenic, mercury, copper and manganese generally reflected the treatment differences, suggesting significant uptake potential from Oldham tailings and contaminated soils. Arsenic and mercury levels were also very typical of those documented in samples collected during the 1987 and 1988 field surveys of the vegetation (Lane et al., 1988, 1989; see Section 3.4). Cadmium and zinc levels were high in the control plants because of the Pro-Mix soil, and hence significant treatment differences were only evident upon comparison of control and 100% tailings treatments (high zinc concentrations only noted in root tissues). Aluminum, iron, and lead concentrations in the roots and shoots were not significantly different amongst the five treatments.

3.3 Growth and Metal Uptake by Commercial Grass Species and Some Considerations for Tailings Reclamation

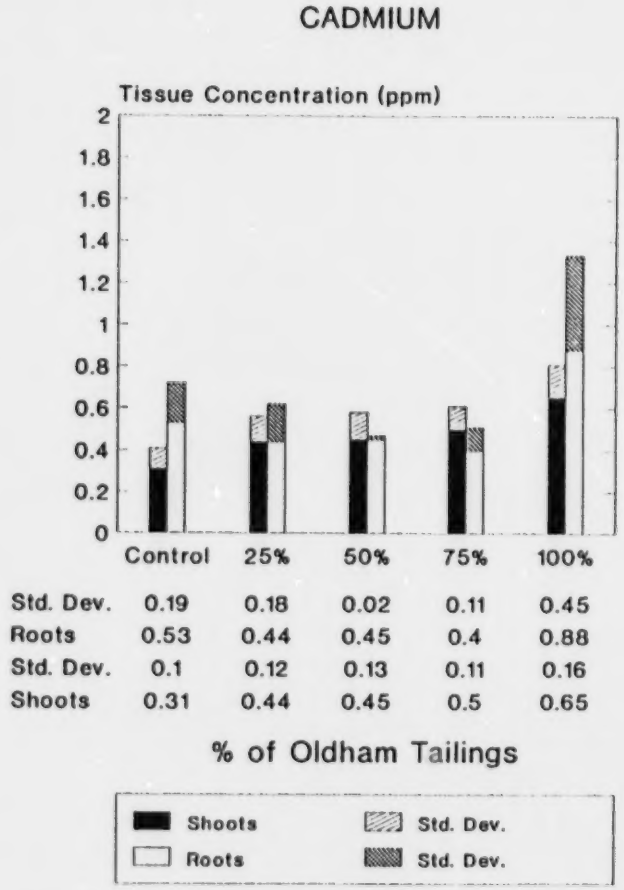
All six species of commercially-available seed germinated and began to grow on the Oldham tailings sample. Subsequent growth, however, was quite variable depending upon liming application and initial fertilizer used (Figures 3.3-1 to 3.3-3; Appendix 8). Of the four grasses and two legumes, only Canada Bluegrass *Poa compressa* appeared to be negatively affected by the tailings. Application of a high phosphorus fertilizer (10:52:10 % mix of nitrogen: phosphorus:potassium) appeared to alleviate this problem. This practise also markedly increased the growth rates of the two fastest-growing species, Creeping Red Fescue *Festuca rubra* and Red Top *Agrostis gigantea* (limed treatments only). As noted earlier, phosphorus amendments appear to assist in development of tolerance to arsenic, and presumably other heavy metals (Benson, 1953; Macnair and Cumbes, 1987). Although the mechanisms for

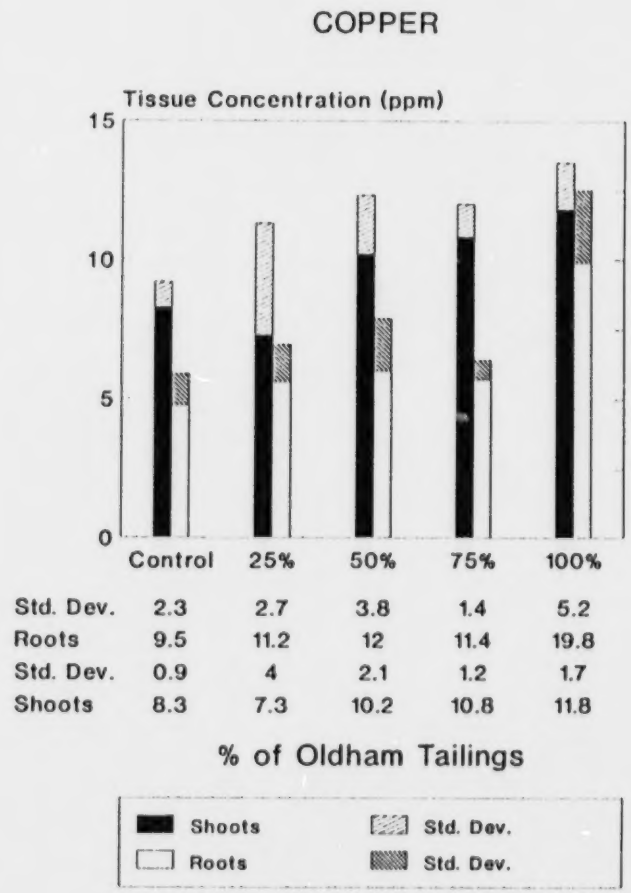


Average growth rate of shoot tissues of the rush *Juncus articulatus* during seven weeks incubation of the plants in pails containing silica sand, Oldham tailing, or 25:75, 50:50 and 75:25 % mixtures of sand:tailings.

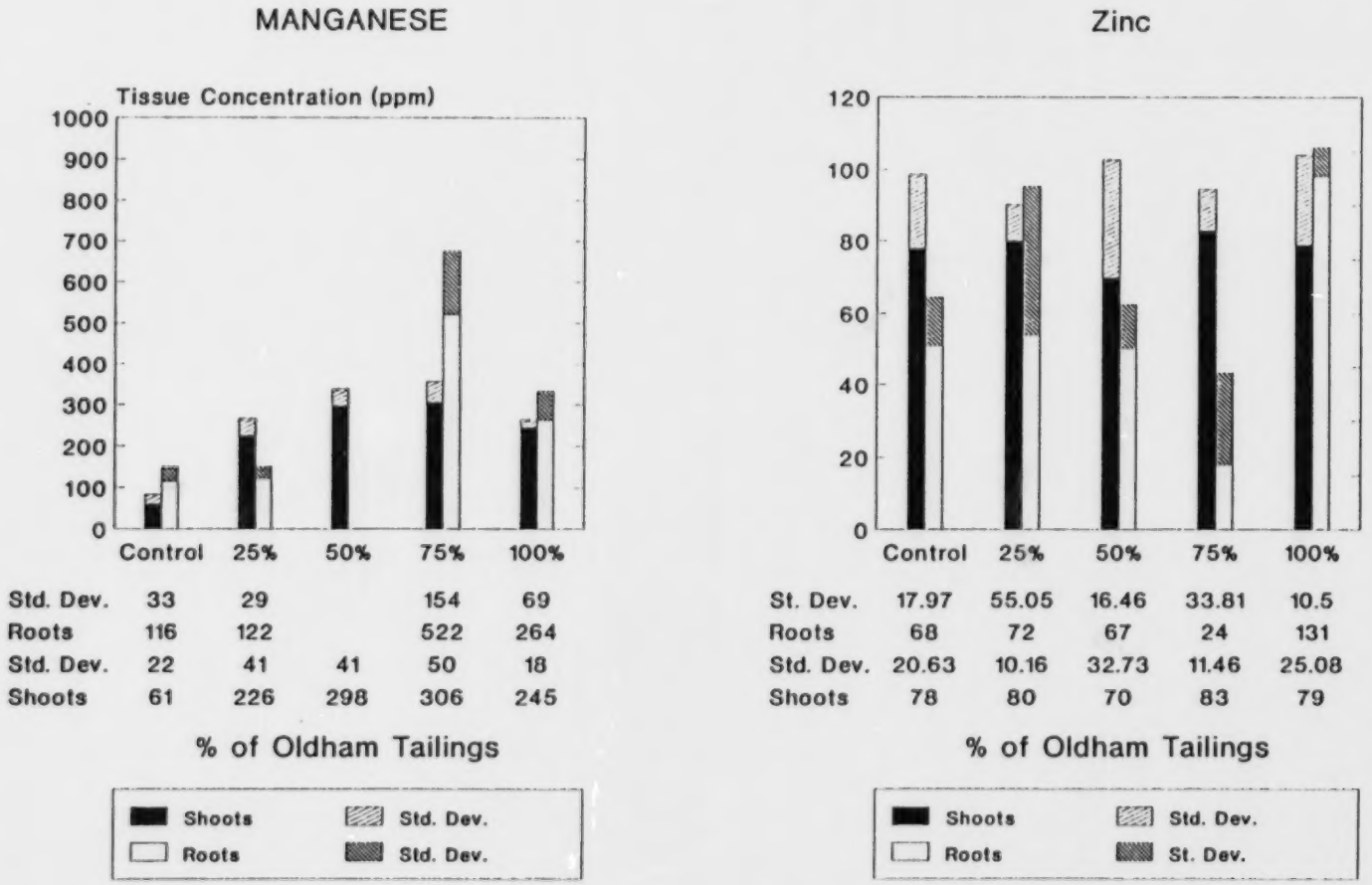


Arsenic and mercury concentrations in the shoot and root tissues of the *Juncus articulatus* after seven weeks growth in pails containing sand, Oldham tailings or 25:50, 50:50, or 75:25 % mixtures of sand:tailings.





Cadmium and copper concentrations in the shoot and root tissues of the *Juncus articulatus* after seven weeks growth in pails containing sand, Oldham tailings or 25:50, 50:50, or 75:25 % mixtures of sand:tailings.



Manganese and zinc concentrations in the shoot and root tissues of the *Juncus articulatus* after seven weeks growth in pails containing sand, Oldham tailings or 25:50, 50:50, or 75:25 % mixtures of sand:tailings.

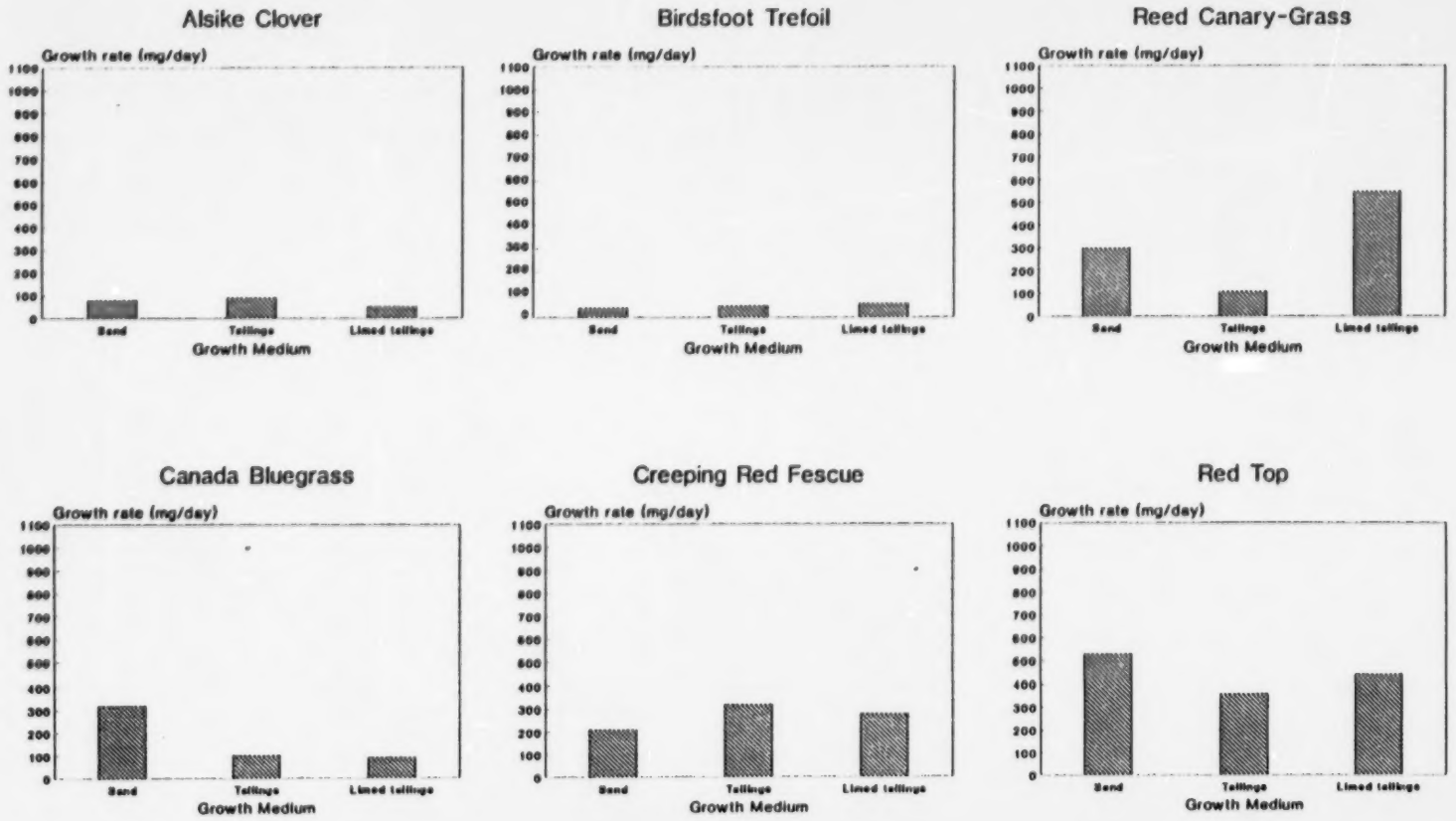
detoxifying and sequestering heavy metals within plants are still unclear, it appears that large amounts of phosphorus are required to produce (a) the enzymes that regulate the permeability of the roots (acid phosphatases), and (b) a class of metal-binding peptides, termed "phytochelatins" (Harborne, 1988).

High nitrogen amendments, by contrast, are not recommended according to these preliminary experiments. The growth of Canada Bluegrass, Reed Canary-Grass *Phalaris arundinacea* and Red Top were all sharply reduced in both control (silica sand) and tailings treatments. The other three species, Alsike Clover *Trifolium hybridum*, Birdsfoot Trefoil *Lotus corniculatus*, and Creeping Red Fescue, were not affected by this treatment.

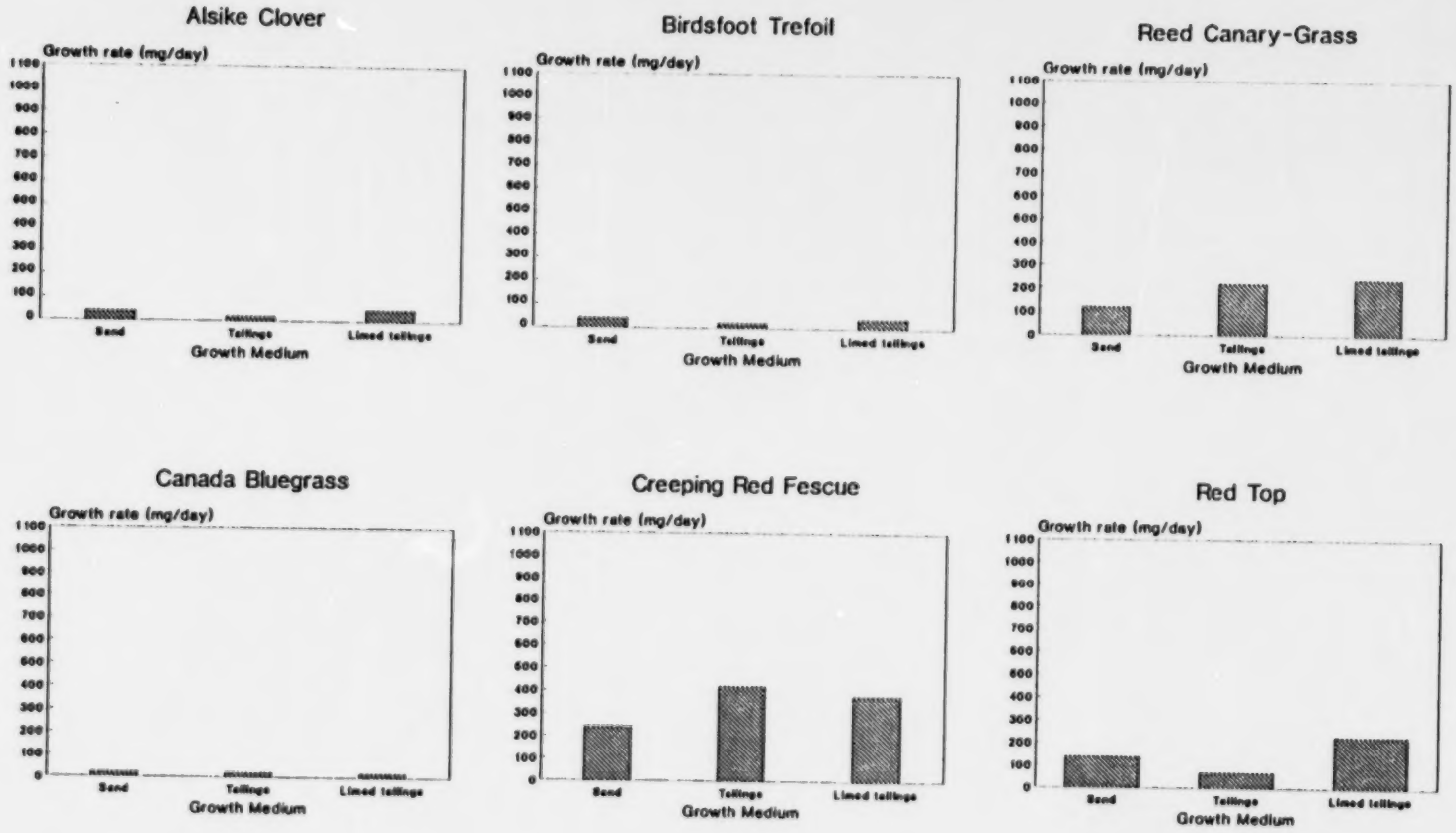
Liming of the tailings was particularly important for stimulating growth of all four grass species in trays amended with the high phosphorus fertilizer. Only Reed Canary-Grass seemed to do better under all types of nutrient amendment and liming. Clearly, both liming and high phosphorus fertilizer additions should be advocated at other sites requiring reclamation of bare, acidic tailings. Although the legumes were unaffected by liming, addition of high phosphorus fertilizer did seem to suppress the growth of Alsike Clover. Consequently, the frequent blending of this species with various grass species in commercially-available, reclamation seed-mixes does not seem warranted for mine sites with similar types of tailings to those at Oldham. Birdsfoot Trefoil would be a better alternative legume. Informed choice of reclamation species and their growth requirements can obviously save mining industries and governments both money and time.

As rainfall rapidly percolates through exposed tailings, reclamation schemes also need to consider possible ways of retaining moisture for the developing seedlings. Although most of these species are very tolerant of drought, short dry periods may still kill off many of the seedlings. Possible measures might include seeding early in the spring prior to summer droughts, covering the seeds with a mulch (hydro-mulching operations; also reduces losses to birds), and/or containing the tailings pile and flooding as much of the tailings as possible by damming streams passing through the deposit. All of the species used in the aforementioned experiments survived on continuously-wet tailings. Some of them, including Red Top, Reed Canary-Grass and Canada Bluegrass, are commonly observed in wet meadows, and occasionally in standing water (Fassett, 1957).

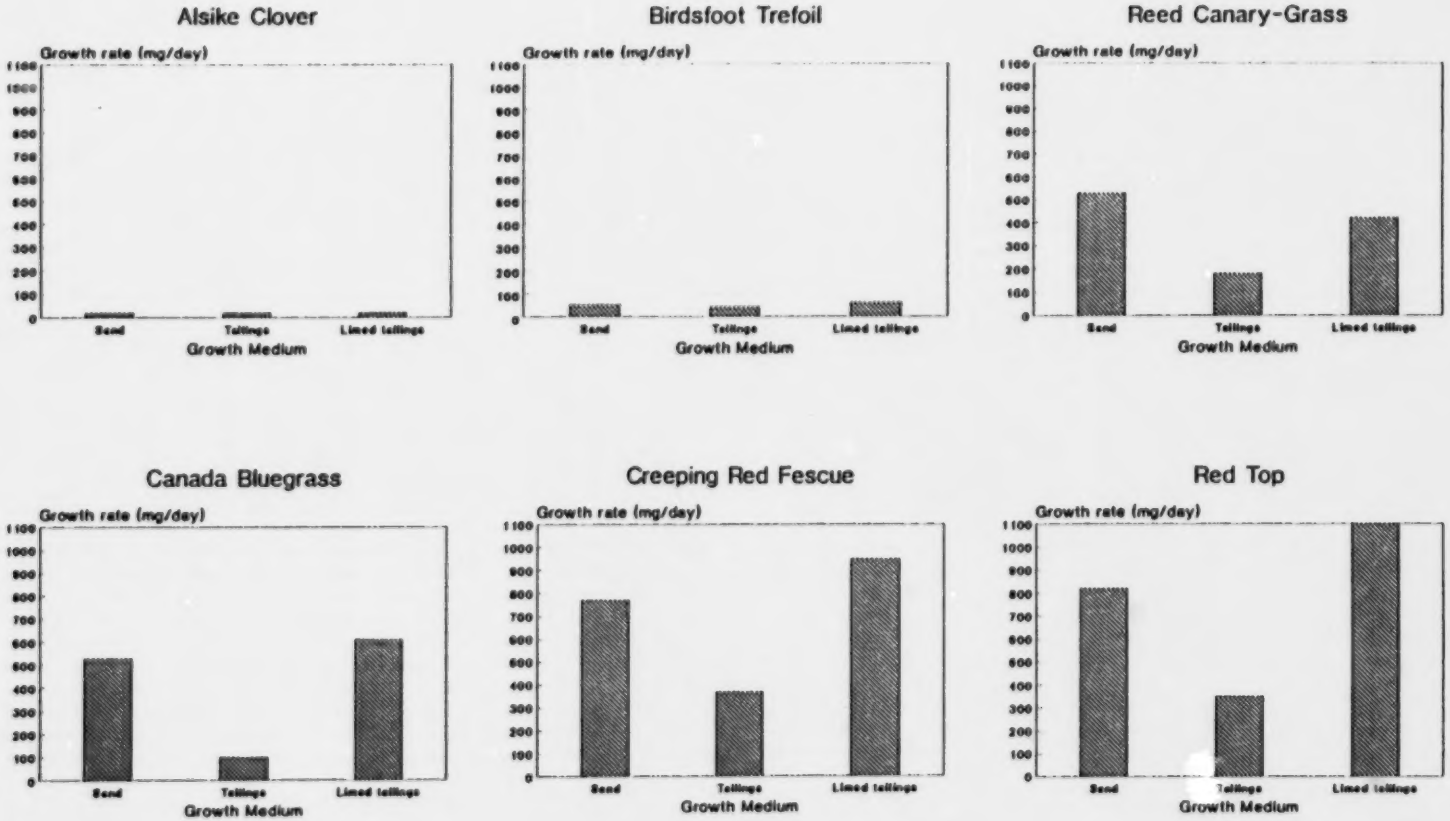
Like growth rates, metal burdens in the shoots of the grass species growing on tailings were also highly variable and dependent upon liming and nutrient treatments (Figures 3.3-4 to 3.3-12; Appendices 9 to 12; insufficient material for any analyses on legume shoots). Lacking sufficient shoot material for all species and treatments (and independent replicates of the nine treatments), it is not easy to discern common trends in this data. Creeping Red Fescue was the only species with sufficient shoot material from all nine treatments. Addition of the high phosphorus fertilizer seemed to decrease its uptake of arsenic, cadmium, copper, lead and zinc. Liming also seemed to be responsible for lowering the tissue levels of arsenic, manganese, cadmium and copper, often to values that were recorded by plants growing in "clean" sand. Similar



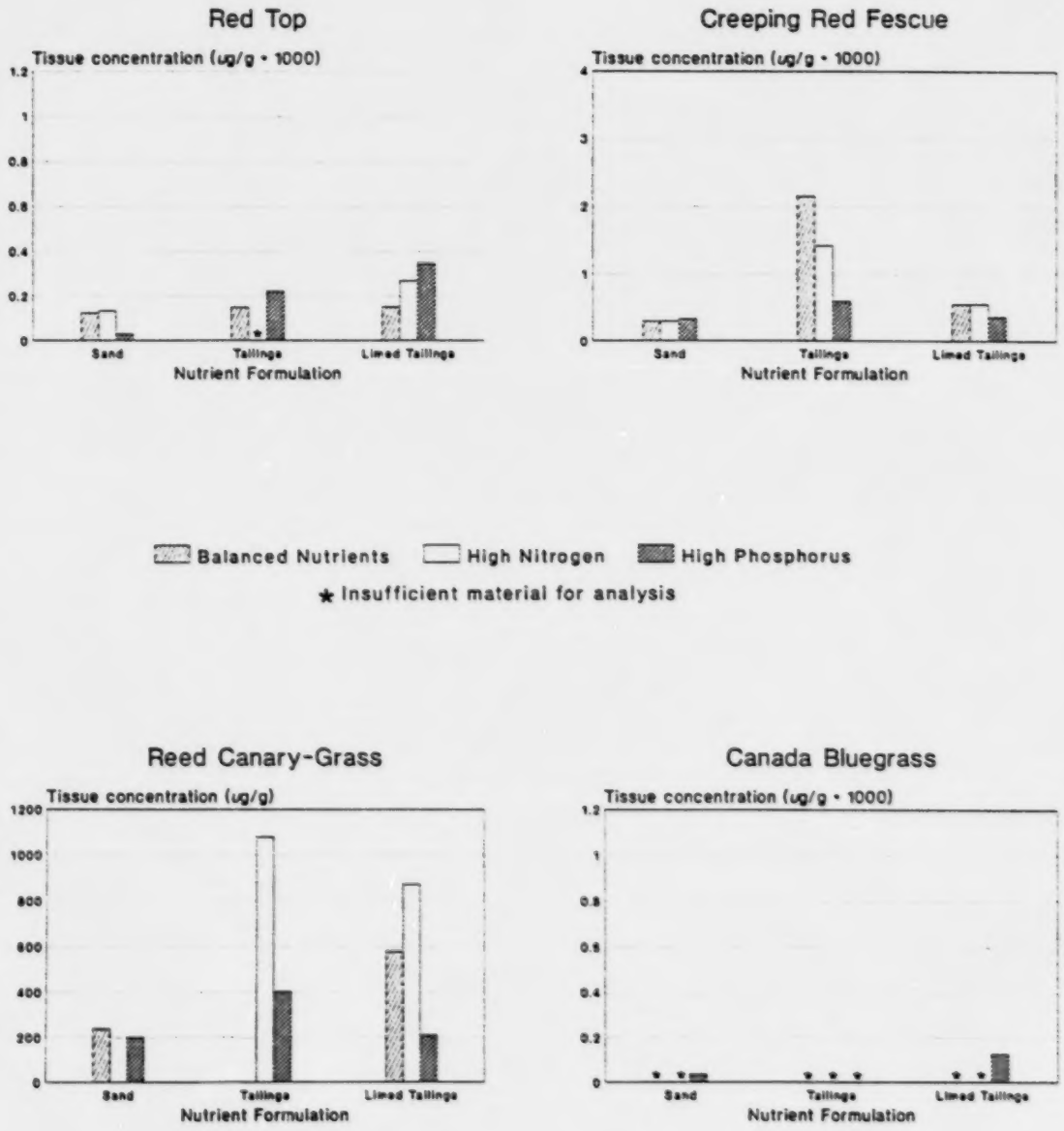
Average growth rates of shoot tissues of Alsike Clover *Trifolium hybridum*, Birdsfoot Trefoil *Lotus corniculatus*, Reed Canary-Grass *Phalaris arundinacea*, Creeping Red Fescue *Festuca rubra*, and Red Top *Agrostis gigantea* that had grown for six weeks in experimental trays or limed tailings, and a balanced, medium-level fertilizer.



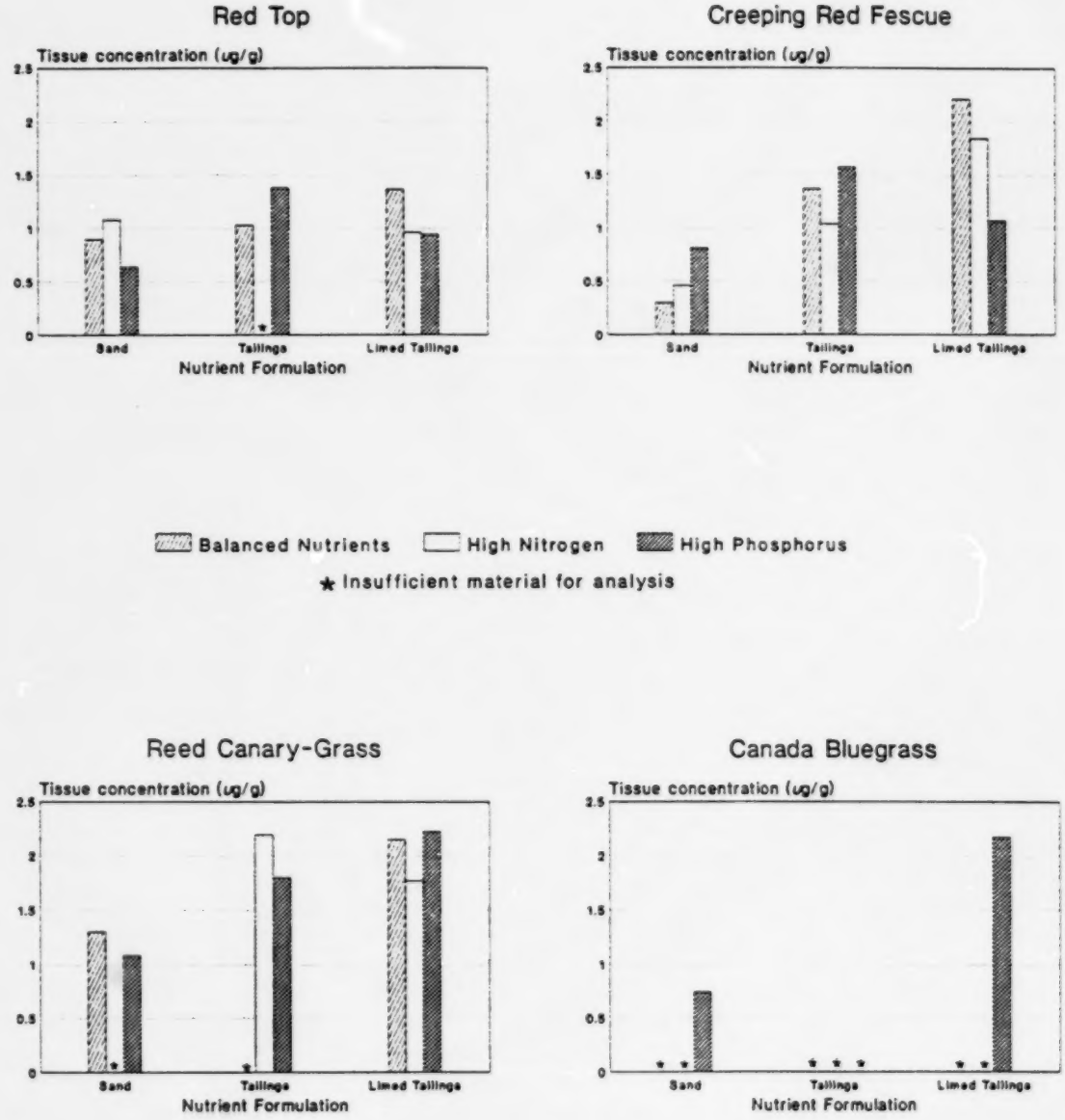
Average growth rates of shoot tissues of Alsike Clover *Trifolium hybridum*, Birdsfoot Trefoil *Lotus corniculatus*, Reed Canary-Grass *Phalaris arundinacea*, Creeping Red Fescue *Festuca rubra*, and Red Top *Agrostis gigantea* that had grown for six weeks in experimental trays or limed tailings, and a high nitrogen fertilizer.



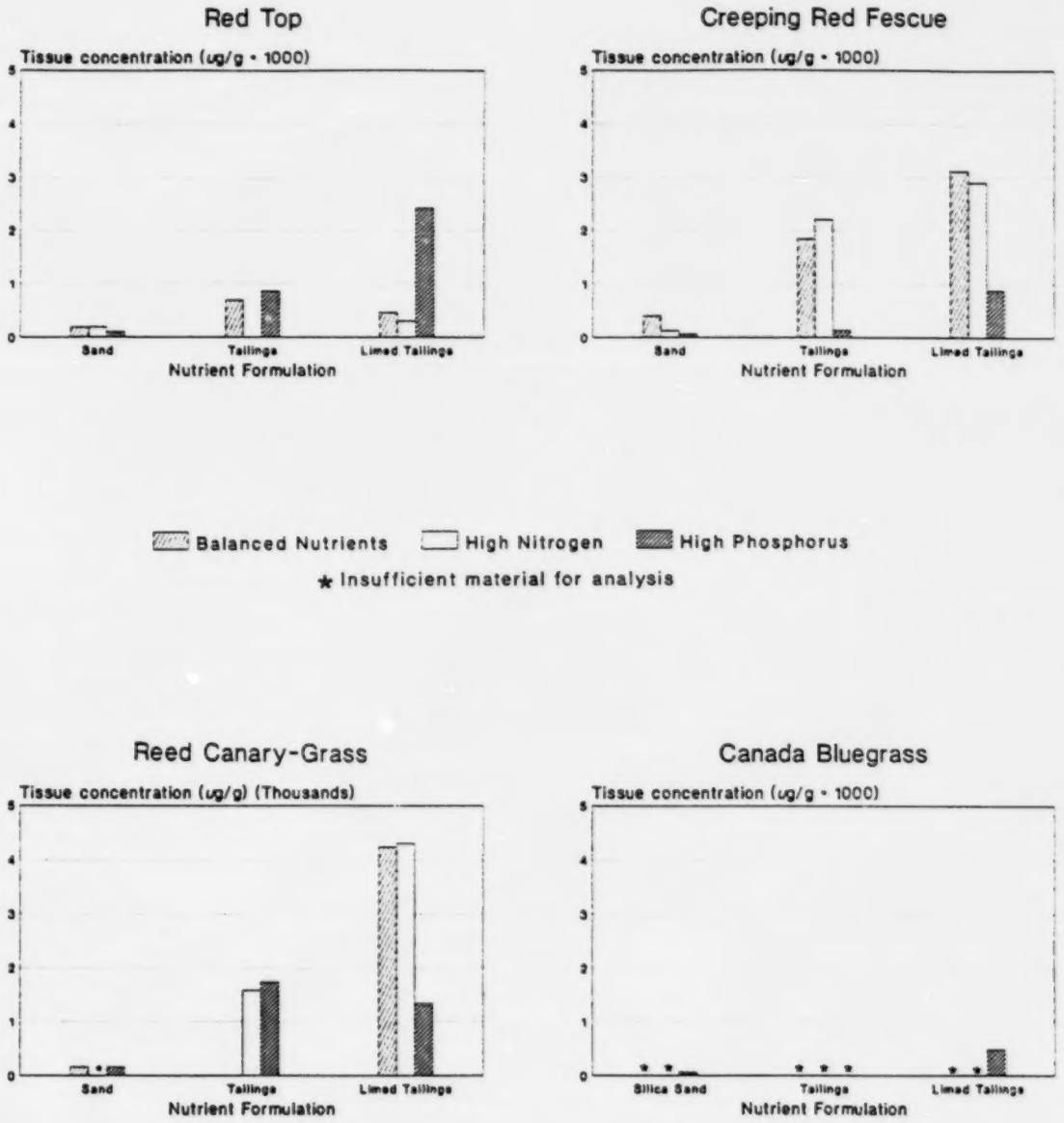
Average growth rates of shoot tissues of Alsike Clover *Trifolium hybridum*, Birdsfoot Trefoil *Lotus corniculatus*, Reed Canary-Grass *Phalaris arundinacea*, Creeping Red Fescue *Festuca rubra*, and Red Top *Agrostis gigantea* that had grown for six weeks in experimental trays or limed tailings, and a high phosphorus fertilizer.



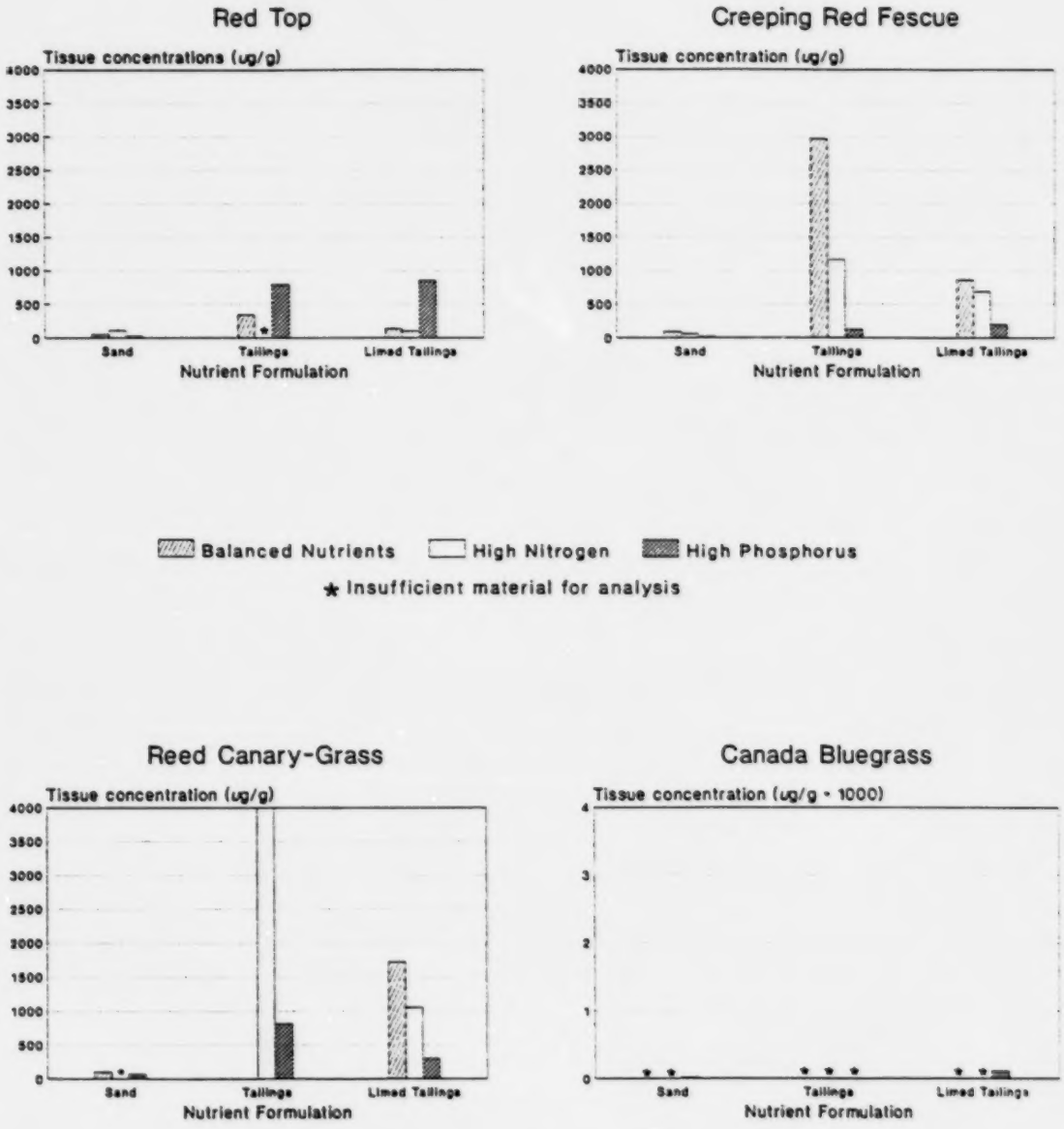
Arsenic concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.



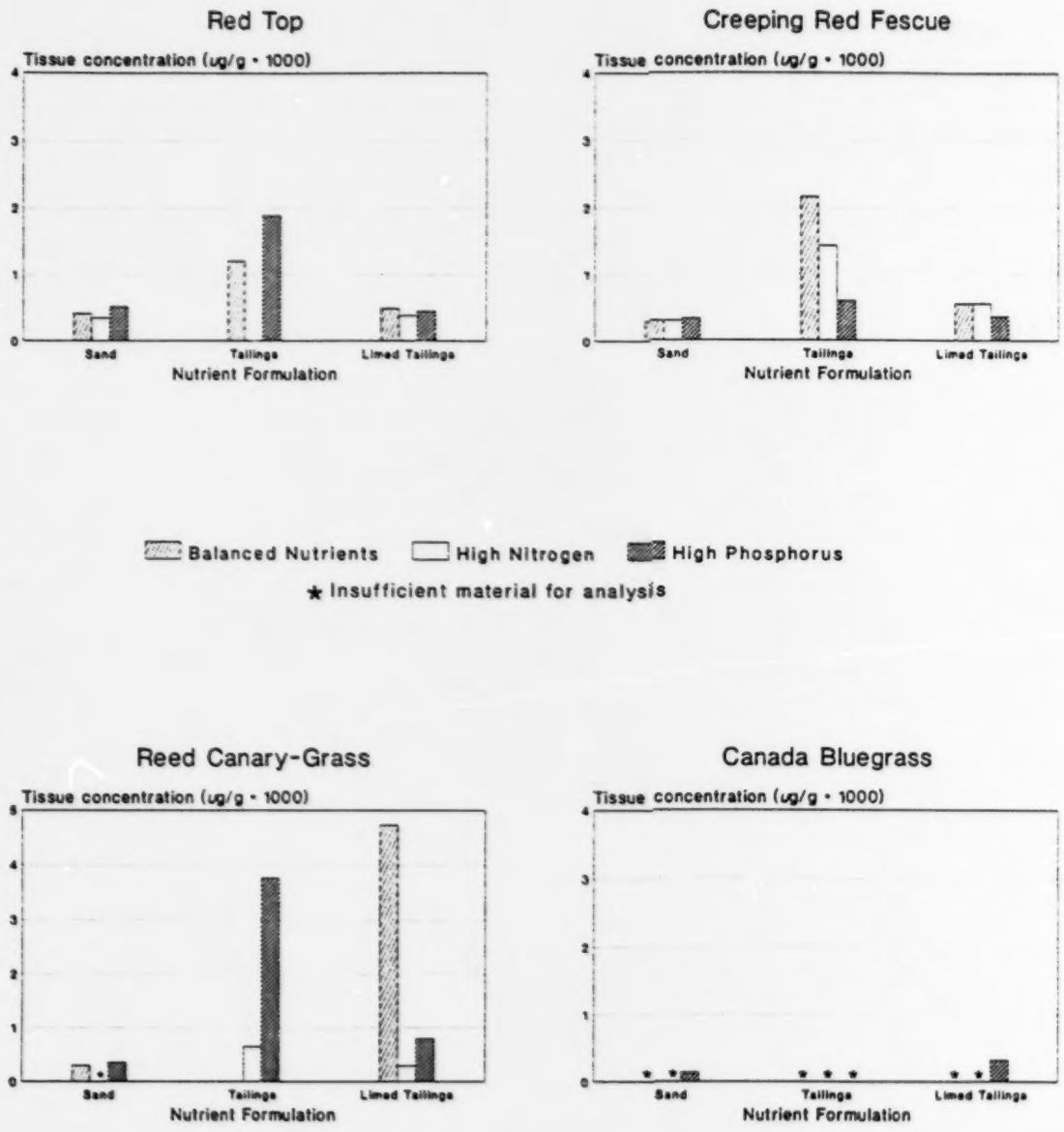
Mercury concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.



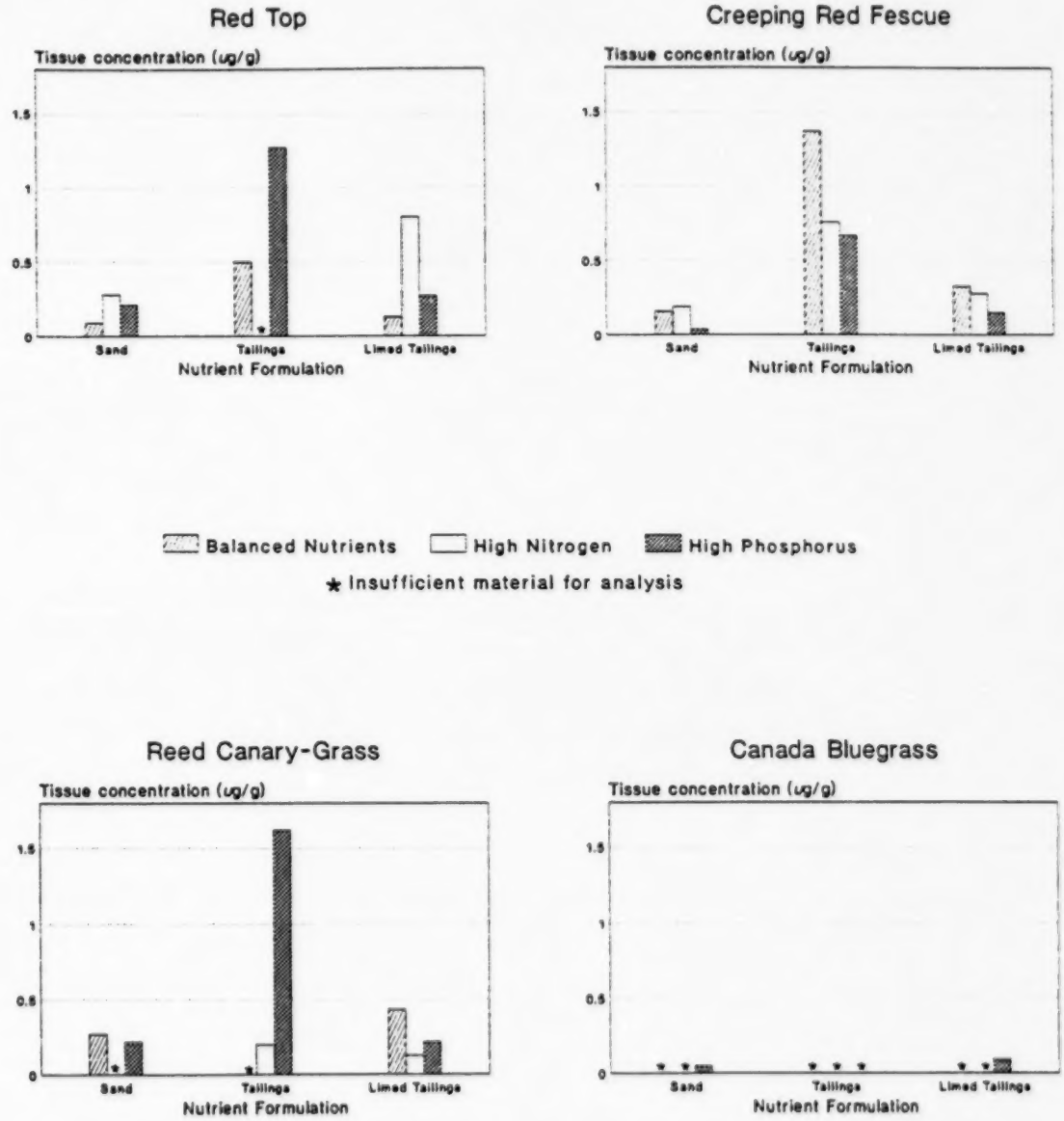
Iron concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.



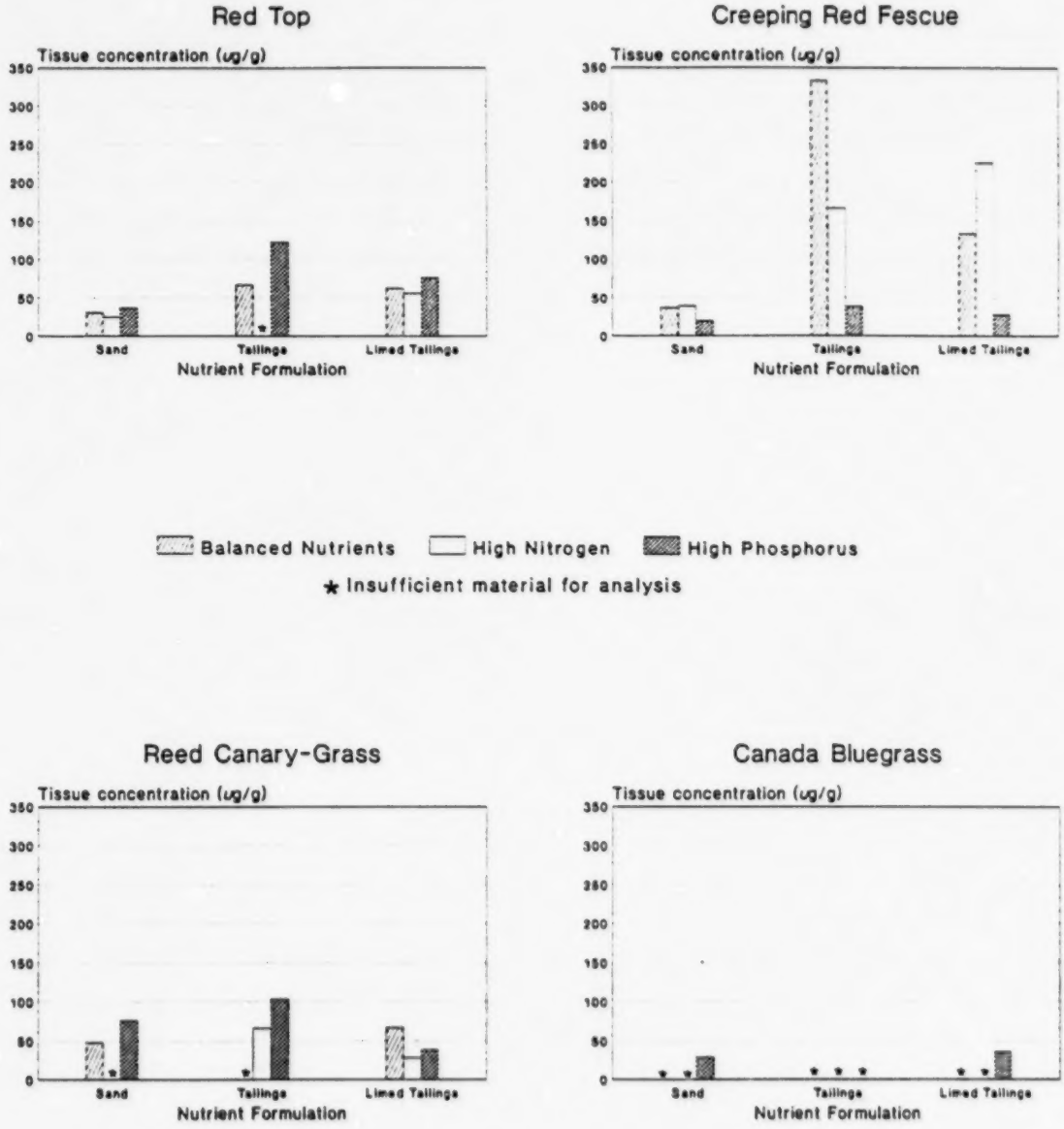
Aluminum concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.



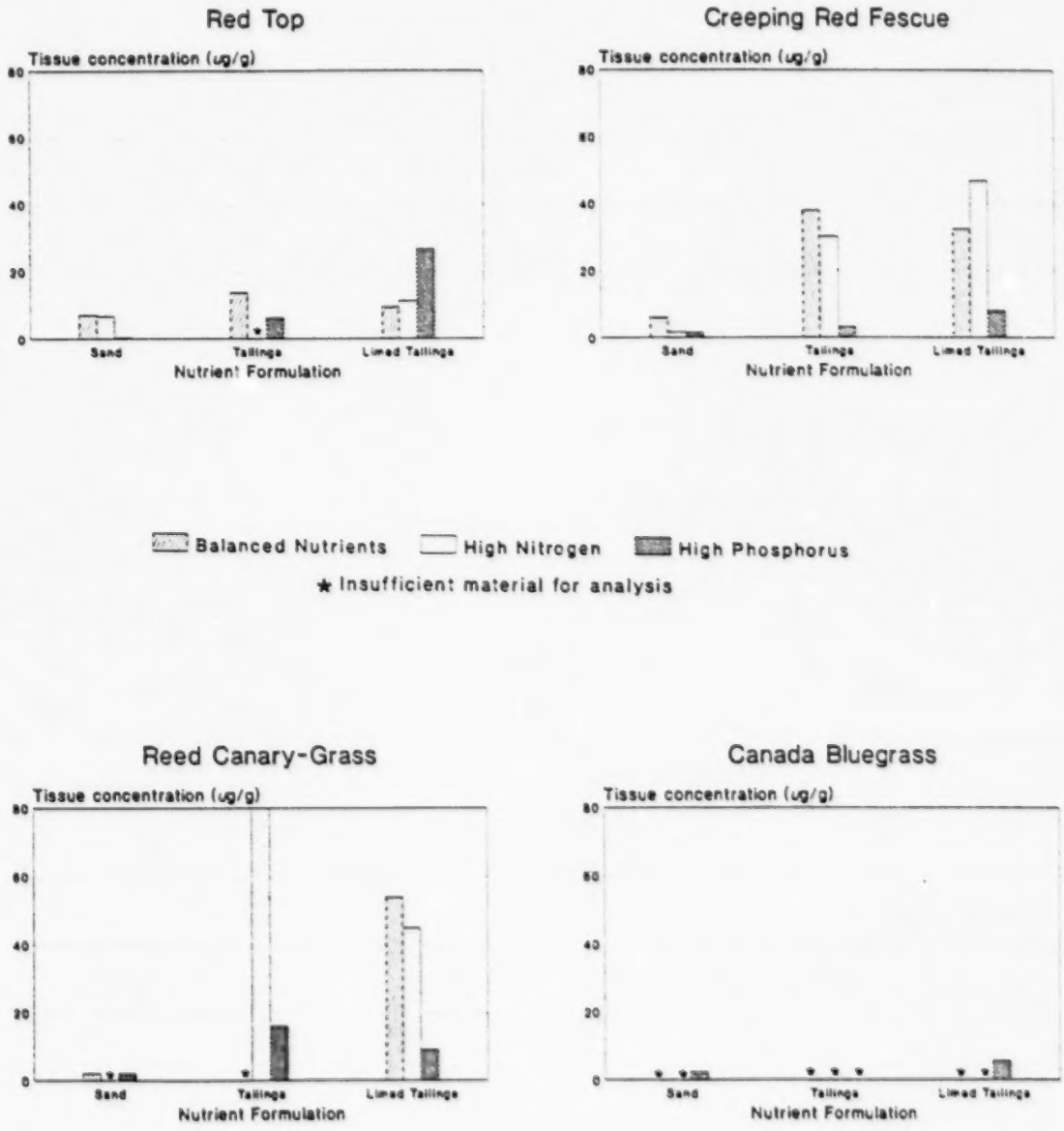
Manganese concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.



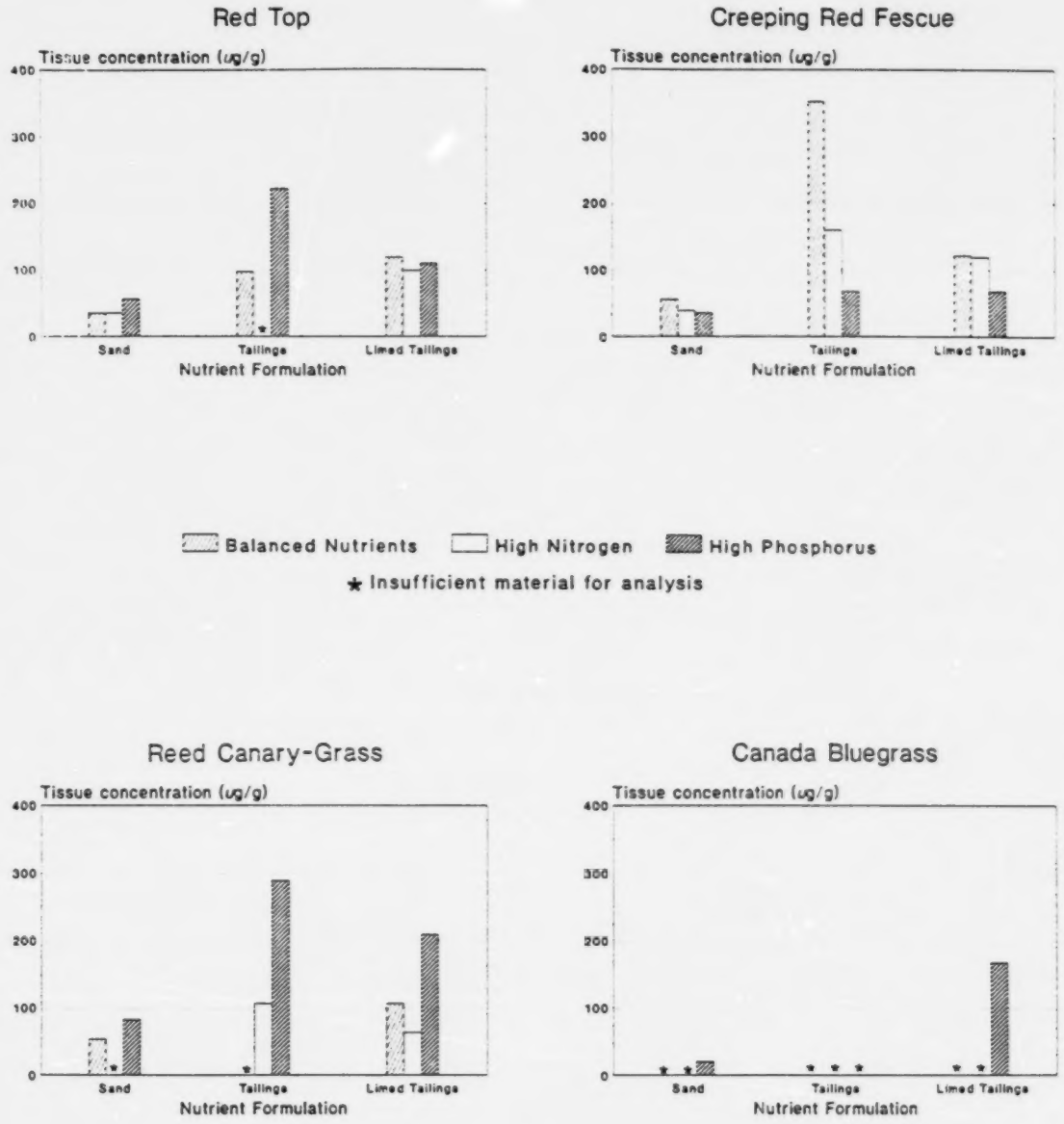
Cadmium concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.



Copper concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.



Lead concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.



Zinc concentrations in the shoots of Red Top *Agrostis gigantea*, Creeping Red Fescue *Festuca rubra*, Reed Canary-Grass *Phalaris arundinacea*, and Canada Bluegrass *Poa compressa*, that had grown, for six weeks, in experiment trays containing silica sand, Oldham tailings or limed tailings, and one of three nutrient formulations, balanced nutrient mixture, high nitrogen or high phosphorus mixtures.

effects on the other three species were only noted for the liming treatment (manganese, cadmium and copper only).

3.4 Field Investigations

All of the surviving plants recovered from the field had similar metal burdens in their shoot tissues as recorded from field collections taken in 1987 and 1988 (Tables 3.4.1 and 3.4.2; Appendix 13; Lane et al., 1989). Metal uptake was apparently quite rapid, attaining the previously documented levels within five weeks of transplantation (Table 3.4.1). Ten weeks incubation did not significantly increase metal levels in any of the plant species. The large ranges in tissue concentrations were most likely a consequence of small-scale variations in soil metal levels and differential growth rates of the plants. Growth of the plants in the field also varied considerably in both time and space (see Table 3.4.1 and Appendix 13), and presumably reflected changes in water availability around the site. That the lab-grown plants also had similar metal burdens (particularly arsenic, see Table 3.4.2), further suggests plants must be constantly incorporating the metals during growth. After death, the metals within plant tissues may gradually return to the soils through direct leaching of dissolved metallo-organic materials, and/or microbial degradation of the shoot and root tissues.

As noted in earlier studies, the moss *Campylium stellata* had the highest concentrations, up to 4000 ppm arsenic and 7.7 ppm mercury (see Table 3.4.2 and Appendix 13). Some unknown fraction of these metals are probably fine, metal-rich particles trapped within the dense growing mat of shoot filaments. High levels in this species may also result from the binding of dissolved metals onto the exterior surfaces of the growing shoots, presumably onto epiphytic algae and bacteria (Bishop and DeWaters, 1988). By comparison, the other three species, horsetails, rushes and cattails, accumulated about an order of magnitude less metal per unit weight than did the moss. Although field trials with grass species were not carried out, grasses also appear to be efficient accumulators of metals, especially in drier areas of the tailings deposits where they form the balance of the vegetation communities (see Table 3.4.2; Lane et al., 1988, 1989).

The prolonged drought in the summer of 1989 clearly demonstrated that adequate water supplies are a key determinant of the success of plant communities. Most of the transplants were in poor condition, or dead, at the end of the field trials. Much of the indigenous vegetation was also largely "burnt-off." Until plants re-establish themselves, late summer and fall rainfall events can easily transport large quantities of metals to downstream environments, and stress many other plants and animals. Clearly, any future use of indigenous plants as "biological filters" for metal-contaminated waters draining mine tailings must take the weather factor into account. Simple plantings will not be the answer to mine-drainage problems; plans must include facilities for retaining waters on-site and continuously flushing the developing plant communities. Such a strategy is embodied within the so-called "constructed wetland" approach for treatment of runoff-water from mines and other areas with exposed, metal-rich bedrock (e.g. Kleinmann, 1987; P. Lane and Associates Limited, 1989). Surface water enters a head-pool and is slowly introduced into a pit or trough containing a man-made wetland. Some of the heavy metals

are incorporated into wetland plants, but most are fixed in the sediments by chemical precipitation reactions with microbially-generated hydrogen sulfide (Sencindiver and Bhumbla, 1988). Plant communities are primarily used to provide a continuous supply of labile organic matter for the sediment microbes. They may also be used as final "polishers" of the effluent leaving the wetland. Indeed, if on-going investigations into the feasibility of organic compost-based systems are successful (Bechard et al., 1989; Kleinmann, 1990), future bioremediation efforts may utilize a final polishing step only when regulations require a very "clean" effluent.

TABLE 3.4.1.

A comparison of growth rates and metal burdens in tissues of the moss *Campylium stellata*, the horsetail *Equisetum fluviatile*, the rush *Juncus articulatus*, and the cattail *Typha latifolia*, resulting from 36 or 66 day incubations of lab-acclimated plants back in the field. Arsenic (As) and mercury (Hg) concentrations for long and short incubations of the plants are the means and standard deviations of replicate samples (number of experimental pots).

Plant Species	Growth Rate (mg/day)		As Concentration (ppm)		Hg Concentration (ppm)	
	Short	Long	Short	Long	Short	Long
moss	7.2 ±4.3 (10)	7.9 ±3.1 (10)	390 ±270 (10)	2100 ±1500 (10)	1.6 ±1.0 (10)	3.0 ±2.6 (10)
horsetail	15.5 ± 0.7 (2)	8.6 ±2.3 (10)	80 ±14 (2)	520 ±560 (9)	0.6 ±0.4 (2)	0.6 ±0.3 (6)
rush	11.7 ± 1.0 (5)	8.4 ±1.0 (10)	160 ± 50 (5)	170 ±150 (10)	0.2 ±0.1 (5)	0.2 ±0.04 (10)
cattail	-	6.8 ±1.5 (10)	-	160 ±100 (10)	-	0.1 ±0.01 (10)

TABLE 3.4.2.

A comparison of the arsenic and mercury concentrations in shoot tissues of plants used in field trials and laboratory experiments with those documented from earlier vegetation surveys at the Oldham site (latter data from Lane et al., 1989).

Plant Species	Arsenic Concentration (ppm)			Mercury Concentration (ppm)		
	Lab Expts.	Field Trials	Surveys	Lab Expts.	Field Trials	Surveys
moss *Campylium stellata*	80 - 230	90 - 4000	3 - 1500	7 - 19	0.5 - 7.7	0.6 - 8.9
horsetail *Equisetum fluviatile*	140 - 420	30 - 1600	3 - 700	5 - 20	0.2 - 1.2	<0.1 - 1.5
rush *Juncus articulatus*	10 - 90 20 - 40[1]	30 - 530	3 - 100	1 - 4 0.3 - 0.5	0.1 - 0.6	<0.1 - 0.4
grass[2] *Agrostis* spp.	150 - 220[1]		4 - 400	1.0 - 1.4		0.1 - 0.5

[1] plants grown in Oldham tailings; other lab results from hydroponic growth of plants in a nutrient solution amended with sodium arsenate and/or mercuric chloride

[2] lab experiments used Red Top *Agrostis gigantea*; only metal burden data from field collections of grasses were from *Agrostis palustris*, the Creeping Bent Grass

4. CONCLUSIONS AND RECOMMENDATIONS

1 - Horsetails and cattails appeared to accumulate the highest levels of arsenic and mercury in lab experiments examining the effects of added metals on the growth of moss, horsetail, rush and cattail species. Growth rates, however, were suppressed by the added metals compared to control hydroponic solutions. Because the rush had the fastest growth rates, incorporated moderate levels of metals, and was one of the prime colonizers of tailings at the Oldham site, it was chosen for further lab studies designed to investigate the feasibility of using indigenous plants as "biological filters" for heavy metals.

2 - Growth of the rush was suppressed by ~40 % when grown in experimental pots containing Oldham tailings or mixtures of sand and tailings. Only a small amount of the limiting substance(s) appeared to be responsible for this effect. Metal levels in shoot and root tissues were virtually identical to those observed in previous field surveys at the site.

3 - Commercially available grass and legume seeds germinated and grew on bare, wet tailings from the Oldham site in a third lab study on the feasibility of using commercial seed to revegetate gold tailings. All but one grass species were unaffected by the heavy metals present in the tailings sample. Addition of a high phosphorus fertilizer seemed to alleviate this problem, and when supplied along with horticultural lime, also enhanced the growth rates of the two fastest growing species, Creeping Red Fescue and Red Top. By comparison, applications of high nitrogen fertilizer either had no effect or depressed the growth of three of the four grass species tested. Concentrations of arsenic and mercury in shoot tissues of the four grasses approximated those in a closely-related species collected from earlier field studies at Oldham.

4 - The best revegetation scheme for gold tailings would be to add lime to raise the tailings pH to near neutral levels, fertilize with a high phosphorus fertilizer, and seed with a mixture of Red Top, Creeping Red Fescue, and Birdsfoot Trefoil. Consideration for retaining moisture on-site is required to facilitate survival of developing seedlings.

5 - Field trials of lab-acclimated plants transplanted back to the Oldham site indicate that the weather, specifically rainfall, will be a prime regulator of the success of vegetation communities in scavenging metals from surface and ground waters. Although few of the plants survived the summer drought, metal accumulations in shoot and root tissues were comparable to levels recorded from earlier field collections.

6 - Except for two bare spots, the Oldham site is already completely covered with vegetation communities. Adding more indigenous plants or seeding the site will not greatly reduce the loss of heavy metals to downstream environments. The best solution may be to build an "artificial wetland" and channel water through the sediment portion. Metals are then fixed in the sediments by simple adsorption and by chemical precipitation with microbially-generated hydrogen sulfide.

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6. APPENDICES

Appendix 1. Grain size analyses of silica sand (Nova Scotia Sand & Gravel #0) and Oldham tailings that were used in Laboratory Experiments 1, 2, and 3.

Appendix 2. QA/QC determinations for metal analyses on standard reference vegetation samples (U.S. National Bureau of Standards #1571 - Orchard Leaves; 5 samples analyzed) as performed by the Minerals Engineering Centre, Technical University of Nova Scotia, Halifax.

Appendix 3. Concentrations of arsenic, mercury, copper, lead, manganese, zinc, aluminum and iron in Pro-Mix soil, silica sand, Oldham tailings, and 25:75, 50:50 and 75:25 % sand:tailings mixtures.

Appendix 4. Average growth rates of the moss *Campylium stellata*, the horsetail *Equisetum fluviatile*, the rush *Juncus articulatus*, and the cattail *Typha latifolia*, during seven week incubations of the plants in jars containing control, arsenic-amended, mercury-amended, or arsenic plus mercury-amended nutrient solutions.

Appendix 5. Tissue concentrations of arsenic and mercury (ppm dry weight) in shoots and roots of the moss *Campylium stellata*, the horsetail *Equisetum fluviatile*, the rush *Juncus articulatus*, and the cattail *Typha latifolia* that had been growing in control, arsenic-amended, mercury-amended or arsenic plus mercury-amended nutrient solutions.

Appendix 6. Average growth rates for shoots of rush plants (*Juncus articulatus*) that had been growing for seven weeks in replicate pails containing either silica sand, Oldham tailings, or 25:75, 50:50 and 75:25 % mixtures of sand and tailings.

Appendix 7. Concentrations of arsenic (As), mercury (Hg), iron (Fe), manganese (Mn), aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) in the shoot and roots of rush plants (*Juncus articulatus*) that had been growing for seven weeks in replicate pails containing either silica sand, Oldham tailings, or 25:75, 50:50, and 75:25 % mixtures of sand and tailings.

Appendix 8. Average growth rates for the six legume and grass species that had been growing for six weeks in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen, or high phosphorus mixture).

Appendix 9. Metal concentrations in shoot tissues of Red Top (*Agrostis gigantea*) that was growing in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen (N), or high phosphorus (P) mixture).

Appendix 10. Metal concentrations in shoot tissues of Creeping Red Fescue (*Festuca rubra*) that was growing in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen (N), or high phosphorus (P) mixture).

Appendix 11. Metal concentrations in shoot tissues of Reed Canary-Grass (*Phalaris arundinacea*) that was growing in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen (N), or high phosphorus (P) mixture).

Appendix 12. Metal concentrations in shoot tissues of Canada Bluegrass (*Poa compressa*) that was growing in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen (N), or high phosphorus (P) mixture).

Appendix 13. Average growth rates and metal burdens in shoot tissues of the moss *Campylium stellata*, the horsetail *Equisetum fluviatile*, the rush *Juncus articulatus*, and the cattail *Typha latifolia*, resulting from either 36 or 66 day incubations of lab-acclimated plants back in the field.

Appendix 1.

Grain size analyses of silica sand (Nova Scotia Sand & Gravel #0) and Oldham tailings that were used in Laboratory Experiments 1, 2, and 3.

Grain Size		Percent of Total Weight	
phi units	diameter (mm)	Silica Sand #0	Oldham Tailings
< 0	> 1	4.0	3.3
0 to 1	1 to 1/2	39.1	27.5
1 to 2	1/2 to 1/4	33.2	42.0
2 to 3	1/4 to 1/8	22.9	21.9
3 to 4	1/8 to 1/16	0.5	3.8
> 4	< 1/16	<0.1	1.4

Appendix 2.

QA/QC determinations for metal analyses on standard reference vegetation samples (U.S. National Bureau of Standards #1571 - Orchard Leaves; 5 samples analyzed) as performed by the Minerals Engineering Centre, Technical University of Nova Scotia, Halifax. Units are given as ppm. NV = value not provided.

Metal	Mean	Standard Deviation	Recommended Value
arsenic	10.4	0.6	10 ± 2
mercury	0.150	<0.001	0.155 ± 0.015
aluminum	111	5	NV
cadmium	0.20	0.06	0.11 ± 0.01
copper	12.88	0.18	12 ± 1
iron	160	6	300 ± 20
lead	47.8	1.1	45 ± 3
manganese	92.0	0.7	91 ± 4
zinc	28.6	0.9	25 ± 3

Appendix 3.

Concentrations of arsenic, mercury, copper, lead, manganese, zinc, aluminum and iron in Pro-Mix soil, silica sand, Oldham tailings, and 25:75, 50:50 and 75:25 % sand:tailings mixtures. Values are the means and standard deviations of three replicate samples. Concentrations are given in ppm, except for iron and aluminum which are percents. Cadmium concentrations were also measured, but were consistently below the 0.5 ppm detection limit in all six substrates.

Metal	Pro-Mix	Silica Sand	Tailings	25%	50%	75%
arsenic	5±2	5±2	8100±900	2000±300	3300±400	5400±900
mercury	0.06±0.01	0.02±0.01	5.0±0.5	1.7±1.1	3.9±0.1	4.4±1.7
copper	17±5	2±1	41±3	10±1	25±4	34±8
lead	13±1	<2	160±10	47±5	100±5	110±20
manganese	260±13	2±1	400±30	170±20	300±10	340±40
zinc	58±28	3	94±8	28±3	58±4	72±7
aluminum	2.1±0.1	0.11±0.01	7.5±0.7	2.1±0.08	4.0±0.4	5.2±0.3
iron	0.8±0.1	0.08±0.03	4.2	1.3±0.1	2.8±0.2	3.2±0.2

Appendix 4.

Average growth rates of the moss *Campylium stellata*, the horsetail *Equisetum fluviatile*, the rush *Juncus articulatus*, and the cattail *Typha latifolia*, during seven week incubations of the plants in jars containing control, arsenic-amended, mercury-amended, or arsenic plus mercury-amended nutrient solutions. Growth rates are expressed as mg dry weight/day. A negative value indicates an average decline in tissue weight during the incubation period.

Plant Species	Treatment	Replicate 1	2	3	4	5
moss	control	3.7	2.1	3.5	4.2	1.2
	arsenic	1.5	2.1	2.3	7.1	2.3
	mercury	3.1	1.3	2.3	9.2	5.2
	arsenic + mercury	1.0	2.9	1.0	3.8	1.7
horsetail	control	6.5	8.4	6.0	7.8	4.6
	arsenic	5.0	4.8	4.4	4.6	7.0
	mercury	3.0	1.8	3.0	3.4	2.2
	arsenic + mercury	2.6	1.8	3.2	3.6	1.8
rush	control	17.6	17.0	23.0	26.6	19.7
	arsenic	24.5	19.4	27.2	17.2	20.6
	mercury	23.6	23.9	31.1	29.1	22.8
	arsenic + mercury	29.4	27.7	18.6	24.5	16.4
cattail	control	8.2	22.7	5.2	6.0	8.8
	arsenic	9.1	13.6	12.8	-7.6	19.0
	mercury	-14.3	8.0	-10.2	-17.9	14.9
	arsenic + mercury	-11.0	5.2	-18.2	23.8	2.5

Appendix 5.

Tissue concentrations of arsenic and mercury (ppm dry weight) in shoots and roots of the moss *Campylium stellata*, the horsetail *Equisetum fluviatile*, the rush *Juncus articulatus*, and the cattail *Typha latifolia* that had been growing in control, arsenic-amended, mercury-amended or arsenic plus mercury-amended nutrient solutions.

Plant Species Treatment	Tissue	Metal	Replicate 1	2	3	4	5
Moss							
control	shoot	arsenic	18	52	16	29	15
		mercury	0.51	0.32	0.65	0.42	0.55
arsenic	shoot	arsenic	232	120	108	76	112
mercury	shoot	mercury	11.49	12.60	13.01	6.82	8.27
arsenic + mercury	shoot	arsenic	165	55	155	89	123
		mercury	19.05	10.00	12.57	10.81	7.55
Horsetail							
control	shoot	arsenic	30	105	85	98	121
		mercury	0.49	0.43	0.75	0.36	0.33
	root	arsenic	39	41	46	128	34
		mercury	0.20	0.18	0.19	0.19	0.16
arsenic	shoot	arsenic	353	322	353	342	419
	root	arsenic	72	49	62	60	127
mercury	shoot	mercury	5.29	8.26	19.58	13.36	18.73
	root	mercury	17.04	22.00	26.26	22.16	23.66
arsenic + mercury	shoot	arsenic	138	244	158	157	274
		mercury	6.21	10.83	5.15	5.89	7.76
	root	arsenic	66	169	52	148	104
		mercury	25.47	53.24	26.40	32.05	22.23

Appendix 5. Continued.

Plant Species Treatment	Tissue	Metal	Replicate 1	2	3	4	5
Rush							
control	shoot	arsenic	17	6	18	13	16
		mercury	0.36	0.44	0.37	0.39	0.39
	root	arsenic	15	9	12	12	17
		mercury	0.38	0.34	0.27	0.19	0.29
arsenic	shoot	arsenic	26	72	48	60	14
	root	arsenic	128	120	60	187	76
mercury	shoot	mercury	2.72	1.27	1.47	2.58	2.57
	root	mercury	8.93	8.90	12.54	9.31	8.28
arsenic + mercury	shoot	arsenic	21	47	68	16	92
		mercury	1.16	2.78	3.54	1.27	1.49
	root	arsenic	48	68	114	24	123
		mercury	7.73	14.23	17.62	11.54	9.89
Cattail							
control	shoot	arsenic	8	4	13	9	19
		mercury	0.20	0.25	0.25	0.46	0.38
	root	arsenic	49	23	16	22	15
		mercury	0.16	0.14	0.24	0.09	0.06
arsenic	shoot	arsenic	88	23	45	72	44
	root	arsenic	205	132	160	104	196
mercury	shoot	mercury	8.25	2.73	13.72	7.92	3.49
	root	mercury	72.80	14.33	83.67	47.37	14.17
arsenic + mercury	shoot	arsenic	303	46	125	116	100
		mercury	2.52	3.16	2.80	1.06	2.49
	root	arsenic	330	117	400	360	142
		mercury	51.37	39.02	19.73	25.57	46.07

Appendix 6.

Average growth rates for shoots of rush plants (*Juncus articulatus*) that had been growing for seven weeks in replicate pails containing either silica sand, Oldham tailings, or 25:75, 50:50 and 75:25 % mixtures of sand and tailings. Growth rates are expressed as mg dry weight/day.

Substrate	Replicate	Shoot Growth Rate
silica sand	1	28.7
	2	24.9
	3	32.2
	4	most plants died
	5	17.2
Oldham tailings	1	18.2
	2	12.6
	3	12.0
	4	most plants died
	5	10.7
25:75 % sand:tailings	1	11.9
	2	16.4
	3	most plants died
	4	19.3
	5	14.3
50:50 % sand:tailings	1	11.9
	2	15.6
	3	26.8
	4	11.8
	5	14.2
75:25 % sand:tailings	1	11.0
	2	16.8
	3	15.2
	4	15.7
	5	11.1

Appendix 7.

Concentrations of arsenic (As), mercury (Hg), iron (Fe), manganese (Mn), aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) in the shoot and roots of rush plants (*Juncus articulatus*) that had been growing for seven weeks in replicate pails containing either silica sand, Oldham tailings, or 25:75, 50:50, and 75:25 % mixtures of sand and tailings. Concentrations are expressed as ppm dry weight.

Substrate	Tissue Replicate	As	Hg	Fe	Mn	Al	Cd	Cu	Pb	Zn
silica sand	shoot 1	6	0.13	128	48	116	0.36	8.0	4.0	69
	2	15	0.28	28	88	4	0.44	9.7	3.0	103
	3	7	0.13	94	31	4	0.16	8.2	1.7	48
	4	8	0.11	650	64	44	0.28	8.2	4.1	87
	5	7	0.26	145	75	64	0.32	7.3	1.6	82
	root 1	13	0.22	745	135	632	0.35	7.4	2.2	52
	2	8	0.21	937	139	1045	0.36	8.1	3.1	58
	3	9	0.30	1120	64	1511	0.49	8.1	1.6	90
	4	33	0.28	487	104	455	0.79	11.1	2.1	84
	5	20	0.30	1497	139	845	0.64	22.7	2.9	54
Oldham tailings	shoot 1	21	0.35	63	255	59	0.67	10.6	2.0	70
	2	40	0.41	68	253	81	0.64	10.5	1.6	73
	3	40	0.42	41	224	28	0.73	11.4	2.0	77
	4	32	0.55	107	230	108	0.82	14.6	1.6	96
	5	36	0.51	129	266	88	0.40	12.0	2.0	77
	root 1	7	0.69	113	-	149	0.28	17.9	2.3	-
	2	5	0.40	120	323	110	0.69	16.1	1.8	199
	3	8	0.77	956	323	1148	1.26	17.7	2.2	137
	4	13	0.83	857	219	619	1.38	17.2	1.7	218
	5	6	0.91	322	190	292	0.77	30.0	1.9	99

Appendix 7. Continued.

Substrate	Tissue	Replicate	As	Hg	Fe	Mn	Al	Cd	Cu	Pb	Zn
75:25 % sand:tailings	shoot	1	37	0.41	107	231	44	0.56	11.5	3.1	84
		2	32	0.33	76	183	32	0.40	9.6	2.0	60
		3	96	0.43	151	184	152	0.40	12.4	4.0	77
		4	45	0.30	349	256	56	0.56	9.6	4.2	132
		5	17	0.24	102	274	20	0.28	10.8	2.8	46
	root	1	7	0.24	958	120	1322	0.43	10.8	1.3	67
		2	14	0.86	668	124	697	0.26	9.5	1.3	65
		3	4	0.73	547	93	499	0.38	15.5	2.1	73
		4	14	0.65	611	169	383	0.75	11.7	3.3	100
		5	17	0.46	639	104	417	0.39	8.7	1.8	57
50:50 % sand:tailings	shoot	1	29	0.51	120	350	104	0.56	7.2	1.7	84
		2	40	0.42	217	320	156	0.60	12.8	4.0	76
		3	22	0.38	40	282	17	0.36	9.3	1.7	57
		4	18	0.32	118	241	96	0.40	10.8	1.3	60
		5	44	0.29	300	298	156	0.32	10.8	3.0	75
	root	1	18	0.73	-	-	-	0.44	15.2	1.0	44
		2	38	0.44	-	-	-	0.49	16.7	3.9	119
		3	20	0.44	-	-	-	0.46	10.9	3.8	81
		4	7	0.58	-	-	-	0.44	8.0	2.4	56
		5	11	0.33	-	-	-	0.43	9.1	2.6	35
25:75 % sand:tailings	shoot	1	32	0.53	78	264	44	0.48	13.2	2.4	84
		2	56	0.31	107	365	84	0.36	11.2	1.6	67
		3	25	0.46	124	308	72	0.56	10.4	1.9	72
		4	44	0.28	273	249	308	0.65	9.9	3.4	126
		5	27	0.37	238	346	232	0.44	12.4	1.6	66
	root	1	6	0.42	-	-	-	-	-	-	-
		2	3	0.71	-	552	-	0.25	3.8	2.1	21
		3	16	0.59	-	680	-	0.51	7.0	3.2	35
		4	20	0.36	-	538	-	0.58	7.1	3.3	42
		5	28	0.99	-	316	-	0.40	14.0	2.0	21

Appendix 8.

Average growth rates for the six legume and grass species that had been growing for six weeks in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen, or high phosphorus mixture). Growth rates are expressed as mg dry weight/m^2/day (value x 10 = g/ha/day). <20 = minimal germination and growth.

Growth Medium	Nutrient Formulation	Alsike Clover	Birdsfoot Trefoil	Reed Canary Grass	Canada Bluegrass	Creeping Red Fescue	Red Top
Sand	Balanced	80	40	300	320	210	530
	High Nitrogen	40	40	120	<20	240	140
	High Phosphorus	<20	50	530	530	770	820
Tailings	Balanced	90	50	110	100	320	360
	High Nitrogen	<20	20	220	<20	420	70
	High Phosphorus	<20	40	180	100	370	350
Limed Tailings	Balanced	50	60	550	90	280	440
	High Nitrogen	50	40	240	<20	380	230
	High Phosphorus	<20	60	420	610	950	1100

Appendix 9.

Metal concentrations in shoot tissues of Red Top (*Agrostis gigantea*) that was growing in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen (N), or high phosphorus (P) mixture). Concentrations are given in μg metal/g dry weight of plant (ppm). im = insufficient material for metal analyses.

Metal	Sand			Tailings			Limed Tailings		
	Balanced	High N	High P	Balanced	High N	High P	Balanced	High N	High P
aluminum	57	113	25	345	im	794	136	107	855
iron	189	194	106	688	im	857	468	308	2411
manganese	416	343	516	1196	im	1884	488	380	448
arsenic	123	133	31	148	im	222	151	268	346
cadmium	<0.09	0.28	0.21	0.49	im	1.27	0.13	0.80	0.27
copper	31	25	37	67	im	122	62	56	76
lead	7.1	6.7	0.4	13.8	im	6.3	9.4	11.3	26.6
mercury	0.90	1.08	0.64	1.03	im	1.38	1.36	0.96	0.94
zinc	35	35	56	97	im	222	119	99	110

Appendix 10.

Metal concentrations in shoot tissues of Creeping Red Fescue (*Festuca rubra*) that was growing in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen (N), or high phosphorus (P) mixture). Concentrations are given in μg metal/g dry weight of plant (ppm).

Metal	Sand			Tailings			Limed Tailings		
	Balanced	High N	High P	Balanced	High N	High P	Balanced	High N	High P
aluminum	98	65	20	2962	1165	133	865	690	206
iron	400	134	68	1844	2207	138	3125	2904	877
manganese	296	293	325	2156	1424	597	550	360	363
arsenic	61	31	36	194	265	250	483	494	158
cadmium	0.16	0.19	0.04	1.37	0.76	0.67	0.33	0.28	0.15
copper	37	39	20	333	116	38	133	225	28
lead	6.1	1.7	1.5	37.9	30.1	3.3	32.4	46.7	8.1
mercury	0.30	0.46	0.81	1.37	1.04	1.57	2.21	1.84	1.07
zinc	55	39	35	352	160	68	121	119	68

Appendix 11.

Metal concentrations in shoot tissues of Reed Canary-Grass (*Phalaris arundinacea*) that was growing in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen (N), or high phosphorus (P) mixture). Concentrations are given in μg metal/g dry weight of plant (ppm). im = insufficient material for metal analyses.

Metal	Sand			Tailings			Limed Tailings		
	Balanced	High N	High P	Balanced	High N	High P	Balanced	High N	High P
aluminum	108	im	67	im	4000	810	1721	1051	300
iron	163	im	157	im	1580	1735	4239	4300	1337
manganese	305	im	355	im	646	3757	472	299	802
arsenic	238	im	202	im	1080	405	579	872	211
cadmium	0.27	im	0.22	im	0.20	1.62	0.43	0.13	0.22
copper	48	im	76	im	66	103	67	28	38
lead	2	im	2	im	80	16	54	45	9
mercury	1.30	im	1.08	im	2.19	1.80	2.15	1.77	2.22
zinc	54	im	83	im	106	289	110	64	209

Appendix 12.

Metal concentrations in shoot tissues of Canada Bluegrass (*Poa compressa*) that was growing in experimental trays containing silica sand, Oldham tailings, or limed tailings, and one of three nutrient formulations (balanced nutrients mixture, high nitrogen (N), or high phosphorus (P) mixture). Concentrations are given in μg metal/g dry weight of plant (ppm). im = insufficient material for metal analyses.

Metal	Sand	Limed Tailings
	High P	High P
aluminum	17	109
iron	70	480
manganese	139	318
arsenic	36	127
cadmium	< 0.05	0.09
copper	29	35
lead	2.2	5.5
mercury	0.74	2.17
zinc	20	167

Appendix 13.

Average growth rates and metal burdens in shoot tissues of the moss *Campylium stellata*, the horsetail *Equisetum fluviatile*, the rush *Juncus articulatus*, and the cattail *Typha latifolia*, resulting from either 36 or 66 day incubations of lab-acclimated plants back in the field. The date of transplantation and harvesting of the experimental pots are given as month/day/year (1989).

Plant Species	Transplant & Harvest Dates	Growth Rate (mg/day)	Arsenic Concentration (ppm)	Mercury Concentration (ppm)
moss	07/18/89	7.0	1100	3.8
	08/24/89	2.7	90	0.5
		5.4	300	1.4
		2.7	220	1.1
		4.6	270	1.2
	08/08/89	14.9	300	2.5
	09/14/89	8.0	290	0.5
		5.1	500	1.5
		6.6	350	1.5
		14.6	470	1.9
	07/18/89	3.6	400	1.3
	09/14/89	5.3	450	1.4
		6.2	4000	7.7
		4.7	260	1.6
		9.2	500	0.9
		6.4	3200	1.1
		12.6	3000	2.2
		10.3	3200	6.2
		10.2	3600	1.5
		10.9	2800	5.8
horsetail	07/18/89	14.9	90	0.9
	08/24/89	16.0	70	0.3
	07/18/89	15.2	80	1.2
	09/14/89	7.9	1600	-
		7.6	-	-
		8.0	40	0.6
		7.9	650	0.6
		8.0	130	0.4
		7.6	200	-
		7.9	940	0.2
		7.9	1000	0.5
		7.6	30	-

Appendix 13. Continued.

Plant Species	Transplant & Harvest Dates	Growth Rate (mg/day)	Arsenic Concentration (ppm)	Mercury Concentration (ppm)
rush	07/18/89 08/24/89	10.0 11.4 12.4 12.2 12.4	130 230 130 190 130	0.2 0.3 0.2 0.2 0.2
	07/18/89 09/14/89	9.7 7.3 7.3 8.0 8.5 8.6 9.1 6.8 9.1 9.8	160 230 70 140 530 70 150 300 40 30	0.6 0.3 0.1 0.4 0.1 0.2 0.2 0.2 0.1 0.1
cattail	07/18/89 09/14/89	5.9 6.2 5.5 8.8 5.8 6.2 6.4 7.4 9.9 5.6	690 190 270 50 260 40 130 280 70 500	0.5 0.6 0.4 0.4 0.7 0.2 0.2 0.1 0.2 0.4